

Study of Pro-thrombotic and Pro-inflammatory Factors in Chagas Cardiomyopathy

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Abstract

Background: The relationship between inflammatory and prothrombotic activity in chagas cardiomyopathy and in other etiologies is unclear.

Objective: To study the profile of pro-thrombotic and pro-inflammatory markers in patients with Chagas' heart failure and compare them with patients of non-chagas etiology.

Methods: Cross-sectional cohort. Inclusion criteria: left ventricle ejection fraction (LVEF) < 45% and onset time to symptoms > one month. The patients were divided into two groups: group 1 (G1) - seropositive for Chagas - and group 2 (G2) - seronegative for Chagas. Pro-inflammatory factor: Ultra-sensitive CRP. Pro-thrombotic factors: thrombin-antithrombin factor, fibrinogen, von Willebrand factor antigen, plasma P-selectin and thromboelastography. Sample calculated for 80% power, assuming a standard deviation difference of 1/3; significant p if it is < 0.05. Statistical analysis: Fisher's exact test for categorical variables; unpaired Student's t-test for parametric continuous variables and Mann-Whitney test for nonparametric continuous variables.

Results: Between January and June 2008, 150 patients were included, 80 in G1 and 70 in G2. Both groups maintained the averages of high sensitivity CRP above baseline values, however, there was no significant difference (p = 0.328). The fibrinogen levels were higher in G2 than in G1 (p = 0.015). Among the thromboelastography variables, the parameters MA (p=0.0013), G (p=0.0012) and TG (p=0.0005) were greater in G2 than in G1.

Conclusion: There is no evidence of greater pro-thrombotic status among patients with Chagas disease. The levels of fibrinogen and the MA, G and TG parameters of the thromboelastography point to pro-thrombotic status among non-chagas patients. Both groups had increased inflammatory activity. (Arq Bras Cardiol 2010; 95(5): 655-662)

Keywords: Chagas cardiomyopathy; thrombin; fibrinolytic agents; inflammation.

Introduction

Heart failure (HF) is a syndrome that is endemic in the world. In Brazil, it has a strong socioeconomic impact, due to the high morbidity/mortality. It is the third overall cause and the first cardiovascular cause of hospitalization in our society¹. In pathophysiology, there is prevalence of inflammatory and neurohumoral activation. The latter is closely related to the activation of prothrombotic factors. This may explain, in part, the increased risk of thromboembolism, sudden death, intracavitary thrombosis and stroke among patients with HF, compared to the population in geral^{2,3}. More recently, the mutual influence between pro-

inflammatory and prothrombotic factors was demonstrated⁴. Markers of endothelial injury/dysfunction, platelet activation or activation of the coagulation cascade^{5,6} may be related to inflammatory activation⁷.

In the Chagas cardiomyopathy (CCM), there has been evidence of a tendency for increased inflammatory activity, which may partly explain the worse prognosis. A study conducted in Brazil showed a relationship between the pro-inflammatory and neurohumoral activation and the worse prognosis, including in the CCM⁸.

Another hypothesis to explain this worse prognosis in the CCM would be a possible additional risk for thrombosis. In pathophysiological studies, there was evidence of a significant increase in pro-thrombotic markers in CCM, compared to healthy controls⁹. However, there was no comparison with other HF etiologies. Several case reports, case series and case-control studies, including of autopsies, have reported high prevalence of ischemic findings, such as renal infarction, arterial thrombosis and other thromboembolic events in this

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population¹⁰⁻¹⁴. However, there are no controlled studies for other etiologies of HF in this respect.

Some pro-thrombotic markers have been studied in patients with HF, namely: von Willebrand factor, thrombin-antithrombin complex, D-dimer, tissue factor, p-selectin, among others. Each marker studies a part of the clot formation. The thromboelastography is an important test in the overall evaluation of coagulation and fibrinolysis, integrating cells and coagulation proteins that are not assessed in the usual tests. The analysis of variables can determine the patient's coagulability state. There is a correlation between the postoperative hypercoagulability state determined by thromboelastography and the postoperative thrombotic complications^{15,16}. Its usefulness in patients with HF has not been established.

Thus, we plan to study the profile of pro-thrombotic and pro-inflammatory markers in patients with CCM. This study will also include thromboelastography, in comparison with patients with HF of non-chagas etiologies.

Methods

Cross-sectional cohort study. Between January 14, 2008 and May 31, 2008, 150 patients with chronic heart failure defined by Framingham criteria were consecutively selected from the outpatient clinic, inpatient units, emergency rooms and intensive care units at our institution.

The criteria for inclusion were symptoms of heart failure for more than a month and left ventricle ejection fraction (LVEF) < 45%. Among the patients included, 80 had Chagas disease - Group 1 (G1) - and 70 were non-Chagas patients - Group 2 (G2), defined by the positive result or negative result, respectively, of at least two serological tests (indirect immunofluorescence and ELISA - enzyme linked immunosorbent assay).

The study excluded patients with mechanical valve prosthesis, with diagnosed neoplasia, who were currently in estrogen therapy, with out-of-control infection, who had used a cardiopulmonary bypass in the last two months, with untreated clinical hypothyroidism or hyperthyroidism, who were apparently or confirmedly pregnant, postpartum patients, patients with liver failure or other clinically relevant coagulopathy, with full anticoagulation, clinical history of thrombophilia, congenital cardiomyopathy, concomitant chronic Chagas and ischemic cardiomyopathy (presence of obstructive coronary atherosclerotic disease of $\geq 50\%$ in left main coronary artery or $\geq 70\%$ in other arteries), or patients who had been on hemodialysis for the past two months.

The study was approved by the institution's Ethics and Research Committee, and all patients signed a consent form. There was an individual clinical assessment form for each patient. This form was filled out with clinical, laboratory, radiological, electrocardiographic and echocardiographic data.

The venous blood was collected on beds, in the case of hospitalized patients, and in the clinical analysis laboratory, in the case of outpatients.

The samples used for determining the tests of fibrinogen, thrombin-antithrombin, P-selectin, von Willebrand factor and

thromboelastography were collected in BD Vacutainer™ tubes (draw volume = 4.5 ml), which contained 3.2% of sodium citrate, through peripheral venipuncture, without applying a tourniquet to the limb. The samples for automated counting of platelets were collected in BD Vacutainer™ tubes (draw volume = 4.0 ml), which contained 7.2 mg of EDTA.

The samples used for determining the serology for Chagas and high sensitivity C-reactive protein were collected into dry Vacuette™ tubes containing gel (draw volume = 5 ml), through peripheral venipuncture.

Pro-thrombotic factors

To evaluate the coagulation system, we determined the serum levels of TAT (thrombin-antithrombin complex) and fibrinogen; to evaluate the platelet activation, we used plasma sP-selectin; to detect endothelial injury, we determined the von Willebrand factor antigen and; for an overall evaluation of coagulation and fibrinolysis, we used thromboelastography. All samples, except for the thromboelastography, were centrifuged twice (at 3,000 rpm) for 15 minutes, at 22° C. Then, the samples were frozen in liquid nitrogen at -180° C and stored at -80° C prior to the final determination. The whole procedure (centrifuging twice, freezing and storing) was carried out twice.

The TAT level was determined by the method ELISA (enzyme-linked immunoassay), Enzygnost™ TAT (Dade Behring, Marburg, Germany), with reference ranges from 1.0 to 4.1 $\mu\text{g/l}$. The quantitative determination of fibrinogen in human plasma was obtained by the Classical Clauss Method, equipment AMAX-190, reference number 886-A (Trinity Biotech, St. Louis, MO, USA), with a reference range between 220 and 380 mg/dl. The sP-selectin was determined by the ELISA technique, by using a soluble 'sP-selectin ELISA' Kit (BioSource™), Catalog Number - KHS2021, with reference range between 90 and 290 ng/ml. The von Willebrand factor antigen in human plasma was determined by the classical method of ELISA - enzyme immunoassay (Coergenix Inc, Broomfield, Colorado, USA), with reference range between 50 and 160%.

The thromboelastography samples were analyzed at 37° C, in a time interval shorter than 120 minutes between collection and testing. We used 20 ml of Kaolin (0.006%), 20 ml of calcium chloride (0.2 Molar) and 300 ml of citrated whole blood. The data were analyzed by the TEC™ software from Haemoscope Corporation (Niles, IL, USA), with graphical representation of the formation and subsequent clot lysis.

The following variables were studied in this test:

a) Coagulation time

- R time (time to reach 2 mm of curve amplitude): is the coagulation time measured in minutes. It measures the time elapsed from the beginning of the test until when the production of fibrin is initiated, with reference range between two and eight minutes.

b) Kinetics of clot

- Determination of k (period between 2 and 20 mm of amplitude of the curve): it represents the moment when the clot stabilizes, the fibrin meshwork consolidates and the platelets adhere to the fibrin meshwork, with reference range from one to three minutes.

- Alpha angle (inclination between “r” and “k”): it measures the clot formation speed. It represents the thrombin generation rate and the conversion of fibrinogen into fibrin, with reference range from 55 to 78°.
- c) Strength of the clot
 - MA (maximum amplitude): it is a direct function of the maximum dynamic property of fibrin and platelet bonding via glycoprotein IIb/IIIa, and it represents the ultimate strength of the clot, with reference range between 51 and 69 mm.
 - G parameter (dyn/cm²): it is the algorithmic conversion of the MA (in mm) of the clot into its pressure resistance value (dyn/cm²). While the MA is used to analyze a linear reaction between clot strength and platelet activity, the G does it as an exponential relationship. G is more sensitive to changes in platelet function, with reference range from 4.6 to 10.9 k.
- d) CI (coagulation index): it allows evaluating the patient’s overall hemostatic state. It results from a combination of the kinetic parameters of the clot (r, k and α angle) and the clot strength (MA), with reference range between -3 and 3.
- e) LY 30 (%) and EPL (%): LY 30 represents the clot lysis after 30 minutes and it makes up, together with the EPL (estimated percentage of lysis), the parameters that monitor the final phase of the coagulation process, with reference range from 0 to 8%.
- f) MRTG (Maximum rate of thrombus generation - mm/min): it represents the peak of thrombin generation, with reference range from 5 to 17 mm/min.
- g) TMRTG (Time to maximum rate of thrombus generation - min): it represents the time elapsed to reach the peak of thrombin generation, with reference range between 6 and 12 min.
- h) TG (Total thrombus generation - mm/min): it represents the total area of thrombin generation, with reference range from 584 to 796 mm/min.

Pro-inflammatory factor

The method used for evaluating the high sensitivity C-reactive protein (CRP) was nephelometry (Dade Behring, Marburg, Germany), with reference range of < 1.0 mg/l.

Statistical analysis

The results were tabulated and analyzed by using software Microsoft Excel 2003 (Microsoft Corporation, Seattle, WA, USA). The software used for the statistical analysis was GraphPad Prism™ (GraphPad Software, Inc, USA).

The sample was calculated by assuming that there was an estimated difference between groups of approximately 1/3 of standard deviation for 80% power, considering the two-tailed hypothesis test. This estimate of difference can be considered reasonable, according to the dispersion measured from prothrombotic variables in other studies in patients with HF. A p value < 0.05 was considered to be statistically significant.

Categorical and continuous variables were expressed as percentages and means \pm standard deviation. The clinical, echocardiographic and laboratory variables among the various groups will be compared by using the unpaired Student’s t-test,

for parametric continuous variables, and the Mann-Whitney test for nonparametric variables. The Fisher’s exact test was used to compare proportions.

Results

Characteristics of sample

Table 1 shows the basic characteristics of the sample studied.

It is possible to note that Chagas patients (G1) are significantly younger, have lower body mass index and worse ejection fraction in two-dimensional echocardiography, compared with non-chagas patients (G2). In this group, there is also a higher incidence of smoking addiction, dyslipidemia, diabetes and hypertension and the more frequent use of acetylsalicylic acid, compared to G1.

Prothrombotic variables

Coagulation system

- Thrombin-antithrombin

Table 2 shows the plasma determination of thrombin-antithrombin. In both groups, it is noted that at least 25% of the

Table 1 - Characteristics of sample

Characteristics	G1	G2	P Value
Age	51.02 \pm 1.256	54.88 \pm 0.8787	p=0.0114
Gender	52M / 28F (65%)	48 M / 22F (68.57%)	p=0.7291
BMI	24,32 \pm 0,3960	27,16 \pm 0,6331	p=0.0001
NYHA FC	2.550 \pm 0.09727	2.3 \pm 0.1045	p=0.0818
LVEF	25.25 \pm 0,9324	27.86 \pm 0.8966	p=0.0472
CI	0.6145 \pm 0.0098	0.5904 \pm 0.0098	p=0.0882
Alcoholism	28/80 (35%)	17/70 (24.28%)	p=0.2111
Smoking	33/80 (41.25%)	42/70 (60%)	p=0.0330
DLP	19/80 (23.75%)	36/70 (51.42%)	p=0.0006
DM	5/80 (6.25%)	17/70 (24.28%)	p=0.0023
SH	29/80 (36.25%)	46/70 (65.71%)	p=0.0005
CRF	8/80 (10%)	8/70 (11.42%)	p=0.7971
COPD	3/80 (3.75%)	7/70 (10%)	p=0.1897
Thyroid disease	6/80 (7.5%)	12/70 (17.14%)	p=0.0818
Beta blocker	78/80 (97.5%)	66/70(94.28%)	p=0.4183
ACEI	75/80 (93.75%)	66/70 (94.28%)	p=1.0
Spirolactone	52/80 (65%)	40/70 (57.14%)	p=0.4010
Digital	40/80 (50%)	25/70 (35.71%)	p=0.0988
ASA	23/80 (28.75%)	37/70 (52.85%)	p=0.0043
Diuretics	60/80 (75%)	47/70 (67.14%)	p=0.3658

G1 – Chagas Group; G2 – Non-Chagas Group; BMI – Body Mass Index; NYHA FC – New York Heart Association Functional Class; LVEF – Left Ventricle Ejection Fraction; CI – Cardiothoracic index; DLP – Dyslipidemia; DM – Diabetes mellitus; SH – Systemic Hypertension; CRF – Chronic renal failure; COPD – Chronic obstructive pulmonary disease; ACEI – Angiotensin converting enzyme inhibitor; ASA – acetyl salicylic acid.

determinations are above the baseline value, with a median close to such value. There was no difference between the groups.

- Fibrinogen

The level of fibrinogen was significantly higher in G2 (343.2 mg/dl ± 69.63) compared to G1 (315.0 mg/dl ± 70.41, p = 0.0156), as shown in Figure 1.

Evaluation of platelets

- Automated platelet count

Table 2 shows the automated platelet count. In this count, there was no difference between the groups studied.

- Human sP-Selectin

Table 2 shows the plasma determination of the human soluble P-selectin. This level was below the minimum baseline value in at least 75% of the sample of both groups.

Endothelial evaluation

- Von Willebrand factor antigen

The plasma determination of the von Willebrand factor is shown in Table 2. In the determination of such factor in both

groups, the median was near the upper limit of baseline values, with a large portion of the sample above that.

The thromboelastography variables

The results of the thromboelastography variables are shown in Table 3.

According to Table 3, the determination of maximum amplitude, G parameter and the total generation of thrombin was significantly higher in G2 than in G1.

Proinflammatory variable

Ultra-sensitive CRP

The results of the determinations of high sensitivity CRP are shown in Table 2. The CRP determination increased in both groups compared to the baseline value. However, there was no difference between the two groups.

Discussion

This is the first controlled study to compare the prothrombotic profile of patients with CCM with HF of non-

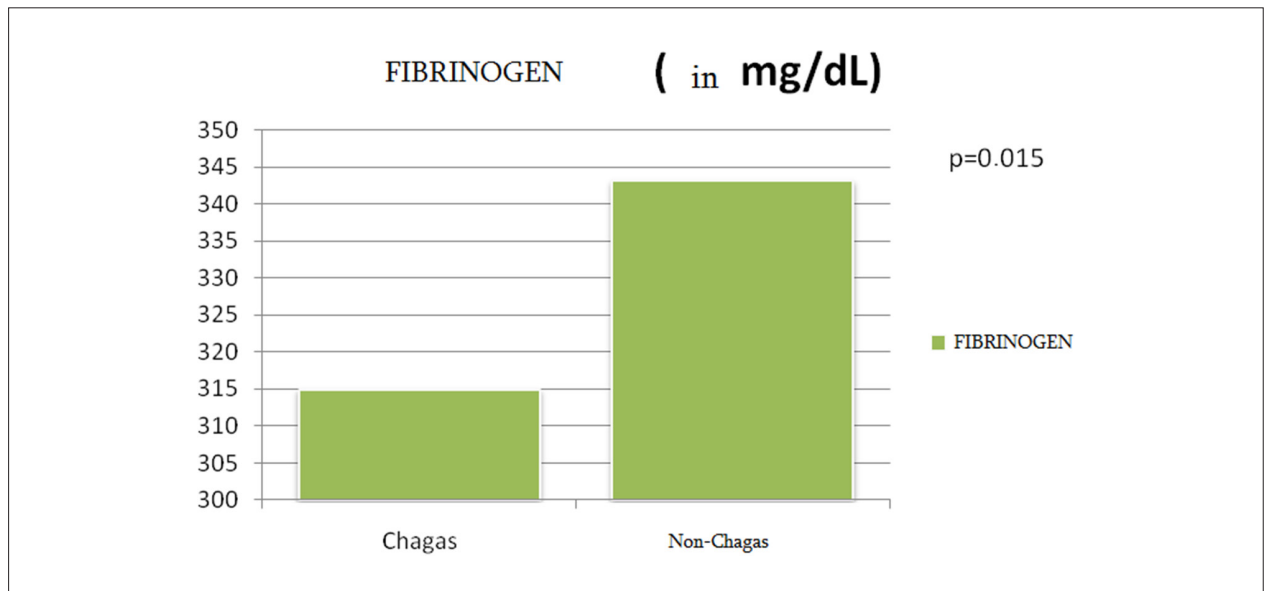


Figure 1 - Plasma levels of fibrinogen among Chagas and non-Chagas patients.

Table 2 - Prothrombotic and proinflammatory variables measured in patients with Chagas and Non-Chagas HF

Variable	G1	G2	P	Baseline value
TAT (mg/l)	3.4(2.4-5.1)	3.5(2.7-4.8)	p=0.2768	1-4.1 mg/l
Platelet count (thousand/mm ³)	202(170-252)	214.5(172-258)	p=0.2609	150-450 thousand/mm ³
sP-Selectin (ng/ml)	46.5(24-81)	46.5(27.75-58.25)	p=0.8248	90- 290 ng/ml
vWF (%)	158(127-232.5)	141(102-181.5)	p=0.0657	50-160%
Hs CRP (mg/dl)	2.04 (0.99-7.88)	3.2 (0.97-8.79)	p=0.3280	CRP < 1mg/l

G1 – Chagas Group; G2 – Non-Chagas Group; TAT - Thrombin-antithrombin complex; vWF - von Willebrand factor antigen; hs-CRP – high sensitivity C-reactive protein; sP-selectin: Soluble P-selectin. All values are expressed as median (interquartile range).

Table 3 - Results of the thromboelastography variables

Variables	G1	G2	P	Baseline value
R time (min)	5.8(4.8-6.8)	6.1(4.9-7.1)	p=0.257	2-8 min
Alpha angle (degrees)	59.4(54.1-64.08)	60.1(53.63-66.3)	p=0.4355	55-78 degrees
MA (mm)	58.90(54.3-69.9)	61.80(58.1-65.25)	p=0.0013	51-69 mm
G (K)	7.2(5.92-8.47)	8.1(6.97-9.4)	p=0.0012	4.6K-10.9 K
CI	-0.5(-1.97-0.37)	-0.2(-1.85-1.0)	p=0.3450	-3 to 3
LY 30(%)	1.450(0.4-3.57)	1.050(0.1-3.125)	p=0.2457	0-8%
MRTG (mm/min)	11.35(9.21-12.89)	11.61(8.9-13.63)	p=0.4137	5 – 17mm/min
TMRTG (min)	7.33(5.94-8.42)	7.585(6.23-8.92)	p=2631	6-12 min
TG (mm/min)	712.2(652.3-762.9)	747.6(706.3-788.3)	p=0.0005	584-796 mm/min

G1 – Chagas Group; G2 – Non-Chagas Group; R Time - Clotting time; MA - Maximum amplitude; G - G Parameter; CI – Coagulation index; LY 30 – Lysis after 30 minutes; MRTG - Maximum rate of thrombus generation; TMRTG - Time to Maximum rate of thrombus generation; TG - Total thrombus generation. All values are expressed as median (interquartile range).

Chagas etiology. With respect to pro-thrombotic markers, we found similarity in most parameters studied and evidence of greater pro-thrombotic activation among non-Chagas patients, as measured by the thromboelastography (G, MA and TG parameters) and as determined by fibrinogen levels. In the analysis of inflammatory activity, we found an increase in such activity, as measured by the high sensitivity CRP, in both groups, which is consistent with previously published studies⁸. And there was no significant difference between the groups.

In the evaluation of the coagulation system, we measured the plasma levels of TAT and the fibrinogen. It is important to measure the TAT because we indirectly measure the generation of thrombin, the central enzyme of the coagulation cascade, which converts fibrinogen into fibrin, stimulates platelet activation and activates factor XIII of coagulation¹⁷. Antithrombin III is the primary inhibitor of thrombin. In pro-thrombotic states, the determination of this complex is expected to rise. In our sample, we found an increase in these levels in more than 25% of subjects in both groups, without any significant difference between the two groups. This demonstrates the procoagulant tendency among chagas and non-chagas patients. Fibrinogen is a plasma protein that is a precursor of fibrin. Thrombin cleaves the fibrinogen to form fibrin monomers, which bond to form insoluble fibrin polymers. It is also an acute-phase protein, whose concentration is increased in response to many different physiological stimuli, such as inflammatory states, infection, trauma, and smoking. High plasma levels of fibrinogen have been associated with pro-thrombotic states. High plasma levels of fibrinogen have also been positively linked with the development of ischemic heart disease, stroke and mortality¹⁸. In the sample studied, the level of fibrinogen was significantly higher in G2 than in G1 (p=0.0156). This may be due to the fact that, in G2, the body mass index is greater (p = 0.0001) and the frequency of smoking is higher (p = 0.0330), compared to G1¹⁹.

The platelet component of the coagulation was assessed by automated counting of platelets and by measuring the level of soluble P-selectin in plasma. The P-selectin is a member of the selectin family of cell adhesion molecules that are

involved with the binding of leukocytes with endothelial cells, platelet-leukocytes, platelet-venules and between platelets. It has been reported that these adhesion molecules may participate in the pathogenesis of HF via modulation of the platelet-leukocyte-endothelium interaction. It is stored in alpha granules of platelets and in Weibel-Palade bodies of endothelial cells. It is rapidly transported to the cell surface under a variety of mediators, including thrombin, histamine and cytokines. It is unclear whether sP-selectin is a causal factor in the formation and progression of atherosclerosis or simply a marker of platelet activation and endothelial damage. Its level may be increased in patients that smoke, patients with diabetes, with ischemic heart disease and with congestive heart failure, which suggests persistent platelet activation^{20,21}. In our sample, both the platelet count and the sP-selectin levels were not different between groups.

The endothelial injury was assessed by checking the plasma levels of the von Willebrand factor (vWF) antigen, which is a plasma protein that is present in the circulation and is bound to factor VIII through a covalent bond. It is synthesized and stored in endothelial cells, but 15-20% is synthesized in megakaryocytes and stored in circulating platelets. The von Willebrand factor may be a marker of endothelial injury and dysfunction, and it may be increased in HF^{22,23}. One study showed a direct relationship between levels of inflammatory markers (IL6), of endothelial injury (von Willebrand factor) and of prothrombotic levels (tissue factor) and decompensation and prognosis of HF. The patients that, after undergoing the treatment of decompensation, improved with a reduction in the levels of these markers had a better rate of survival in a six-month monitoring period⁶. There are no similar data among CCM patients in the literature. The levels of this protein were high in both groups, with a tendency to higher levels in the Chagas group, but without statistically significant differences.

To understand the contribution and interaction of platelets, leukocytes, erythrocytes and plasma soluble components in the coagulation process, special attention has been paid to the generation of thrombin. In a prospective cohort of 914 patients, after the first venous thromboembolic event, the generation of thrombin was able to identify patients at low

risk for recurrence of such events²⁴. Thromboelastography, a technology that allows simulating, *in vitro*, the *in vivo* process of the formation and dissolution of the clot, was first described by Hartert in 1948. This technology has been used extensively for monitoring hemostasis during surgical procedures, in order to guide the transfusion of blood products. The relationship between hypercoagulability measured by TEG[™] and prothrombotic state has been studied. A prospective study noted that the maximum amplitude (MA) measured in the preoperative TEG[™] may be useful for predicting thromboembolic events, with sensitivity of 72% and specificity of 69%. Another study also confirmed that the MA in thromboelastography performed preoperatively predicts postoperative thrombotic complications, including acute myocardial infarction^{15,16}.

Platelets contribute to 80% of the MA, while the fibrinogen participates with 20%, besides providing sites for platelet adhesion. The high MA suggests increased platelet activity, indicating a higher risk of thrombosis or increase in the activity or in the levels of fibrinogen. The G parameter, as it is an algorithmic conversion of MA, is more sensitive to changes in platelet function or fibrinogen levels. In our sample, we found no difference in the number of platelets or in the activation measured by sP-selectin. Therefore, we attribute the difference in G and MA parameters of the thromboelastography to higher fibrinogen levels among non-chagas patients than among patients with Chagas disease. On the other hand, the TG (total generation of thrombin) parameter levels show a pro-thrombotic status that does not depend on fibrinogen, since thrombin is a central enzyme in the coagulation cascade, as it converts fibrinogen into fibrin, which leads to the formation of clot. In our study, the TG was statistically higher in G2 than in G1, $p = 0.0005$. High levels of thrombin generation may represent greater potential for hypercoagulability status²⁵. However, as most of the values of such parameters were within normal ranges in both groups, one might question the clinical importance of such findings.

With respect to pro-inflammatory markers, we studied the high sensitivity CRP, which is one of the main acute-phase proteins. CRP is not only increased in HF, but it is also an independent predictor of cardiovascular mortality, especially among patients with HF of ischemic etiology^{26,27}. The increase in inflammatory activity and worse prognosis in Chagas-related and idiopathic dilated cardiomyopathy⁸. In our study, the determination of CRP was shown to be increased in both groups, but there was no statistical difference between them.

Limitations

The exclusion of patients with full anticoagulation could be selection bias. These patients may have an increased pro-thrombotic profile, since most of them have experienced thromboembolic events or have 'known risk factors' such as atrial fibrillation in most cases. However, there is a small probability that we will alter the therapeutic approach of such patients, because they are already on full anticoagulation. Initially, the purpose of this new line of research is to identify subgroups of patients that are at an increased risk of experiencing such events and, in the future, to study the expansion of indications for anticoagulation in patients with HF.

The proinflammatory variable used in this study was the high sensitivity CRP. Despite the acknowledged use as a prognostic variable in HF, its role in different etiologies of HF is not fully validated. In addition, other pro-inflammatory markers, such as interleukin-6 and tumor necrosis factor-alpha, will be determined at a later time in frozen and stored samples of these patients, in future studies.

The prothrombotic variables studied in this sample were chosen with the purpose of obtaining information about each one of the steps in the clot formation and lysis process. However, other variables that were not addressed in this study could enhance our understanding of other coagulation pathways. There are also plans to establish other prothrombotic factors for this purpose in future studies.

Some readers may miss a healthy control group for comparison purposes. However, as the tests used in this study have been validated and they are widely used in our environment, it would be redundant to use this healthy control group, since we already have reliable baseline values.

Even though the calculation for 80% power is considered adequate, the sample will be expanded to reach 90% power, with an estimated total of 300 patients.

Another limitation of this study is the difference between the groups. G2 is older and more obese than G1. In addition, there is higher prevalence of dyslipidemia, diabetes, hypertension and smoking addiction in G2. Such differences make the two groups mismatched on key variables in the inflammation and thrombosis processes. Thus, differences that may exist between the two groups may have been attenuated by the heterogeneity in the prevalence of risk factors. This effect can be attenuated by statistical resources such as the analysis of covariance.

Finally, it is a cross-sectional cohort study. Thus, it is not possible to obtain any information about the incidence of thromboembolic events in this sample or validation of these markers as prognostic variables in this context. In order to do this, there is a continuation of this study in progress, in the form of a prospective cohort study.

Conclusion

There is an increase in inflammatory activity measured by the high sensitivity PCR in both groups. There is no evidence of higher prothrombotic status in the chagas group, compared to non-chagas patients.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

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