The Prothrombotic State in Early Stages of Chronic Chagas' Disease

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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 β-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 β-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.*

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SEE EDITORIAL ON PAGES 333-4

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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, dímero D y β-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 dímero D ($p < 0,05$). La β-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 dímero D) sugeriría la existencia de un estado protrombótico en estadios tempranos de la enfermedad de Chagas crónica.

Palabras clave: *Chagas. Marcadores. Trombosis. Fibrinólisis.*

INTRODUCTION

Chagas' disease is a histaminic, hematological, parasitic condition caused by the flagellate protozoan *Trypanosoma cruzi*, a blood parasite that dwells and

ABBREVIATIONS

ECG: electrocardiogram.
PIOPED: Prospective Investigation of Pulmonary Embolism Diagnosis.
GOT: glutamate-oxaloacetate transanimase.
GPT: glutamate-pyruvate transanimase.
NV: normal value.
F1+2: fragment F1+2.
MT-AT: modified thrombin-antithrombin.
FDP/fdp: fibrinogen/fibrin degradation products.
ELT: euoglobulin lysis time.
t-PA: tissue plasminogen activator.
PAI-1: tissue plasminogen activator inhibitor-1.
SD: standard deviation.
SE: standard error.

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.[1-3]

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.[1-3]

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.[4]

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.[1]

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.[5-9]

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.[10-12]

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 (according Puigbo et al´s classification13 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[13] (Table 2).

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

TABLE 1. **Demographic data and tests results from patients with Chagas' disease and from control individuals**

Sex, age, test	With Chagas´ disease	Control
Sex		
Men	12	10
Women	30	11
Age, mean±SD	32.5±6.7*	24.2±5.6*
Clinical evaluation	42	21
Functional class		
Ib	31	0
II	11	0
Symptoms		
Ib	11	0
II	0	0
ECG	N:38, A:4	N:21
Radiography	N:42	N: 21
Echocardiogram		
EF	N:42	N:21
WM	N:38, A:4	N:21
Denervation tests	A:42*	N:21*

*$P<.05$. NS indicates not significant; N, normal; A, abnormal; EF, left ventricular ejection fraction; WM, movement of the left ventricular posterior wall.

TABLE 2. **Clinical classification of Chagas' cardiopathy**

	Symptoms	ECG	Cardiac size	Left ventricular ejection fraction	Left ventricular wall motility	Autonomic function
Phase I						
A	None	Normal	Normal	Normal	Normal	Normal
B	None	Normal	Normal	Normal	Slight abnormalities or diastolic dysfunction	May be abnormal
Phase II	Minimal	Conduction abnormalities or extrasystoles	Normal	Normal	Segmental akinesia Aneurysms	May be abnormal
Phase III	Congestive heart failure	Pathological Q conduction abnormalities	Major	Reduced	Overall dysfunction Segmental abnormality of wall motility	Usually abnormal
	Arrhythmia	Complex arrhythmia				

Taken from Puigbo JJ, et al.[13]

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)* petechae 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/m^2; *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 complex[20] (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 method[22]). We considered NV to be up to120 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

TABLE 3. **Thrombotic variables**

Variable	With Chagas' disease (n=42) Mean±SD	Controls (n=21) Mean±SD	*P**
F1+2	4.20 ± 1.52	1.87±0.67	<.0001
ATM	18.53±3.95	13.31±4.45	<.0001
ß-thromboglobulin	29.77±12.04	25.38±10.93	NS
FDP/fdp	5.76±4.08	3.43±0.93	<.05
D-dimer	355.48±139.38	279.86±66.20	<.05

*Mann-Whitney *U* test.

TABLE 4. **Fibrinolitic variables**

Variable	With Chagas' disease (n=42) Mean±SD	Controls (n=21) Mean±SD	*P**
ELT			
Baseline conditions	137.40±32.67	142.86±15.21	<.0001
Stress conditions	70.69±30.09	42.38±7.00	
t-PA			
Baseline conditions	0.40±0.34	0.79±0.25	
Stress conditions	2.30±1.75	3.08±0.74	NS
PAI-1			
Baseline conditions	5.06±3.96	2.66±0.70	
Stress conditions	11.92±6.18	8.15±1.82	NS

*Mann-Whitney *U* test.

(PAI-1) values we used the same methods. The t-PA values under baseline conditions (COASET t-PA, Chromogenix, Milan, Italy) were 0.57 U/mL±0.77 U/mL, and after inducing stress by means of venous occlusion, the cut point for our laboratory was a mean (M) value of 3.08 U/mL±0.74 U/mL. For PAI-1 the NV at baseline (BERICHROM PAI – Dade Behring, Deerfield, Ill.) was 3.1 U/mL±0.2 U/mL, and after stress induced by venous occlusion the cut point for our laboratory was a mean value of 8.15 U/mL±1.8 U/mL.

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. 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

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,[23] 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 thrombotic risk factors.[5] 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.[24] 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.[24] 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, onocological therapy, myocardial infarction, heart failure, and arrhythmia).[25-28]

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.[29] 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.[30] The permanence of risk factors has an important role in thrombotic recurrences.[31]

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 ($P<.0001$), ATM ($P<.0001$), FDP/fdp ($P<.05$), and D-dimer ($P<.05$), shows the existence of a prothrombotic stage in stages IB and II of the Puigbo classification of chronic Chagas' disease.

REFERENCES

1. Milei J, Storino R, Matturri L, Rossi L. Anatomo-clinical and epidemiologic study of Chagas disease. Pathologica 1996;88:117-27.
2. Rassi A, Rassi A, Little W. Chagas' Heart Disease. Clin Cardiol 2000;23:883-9.
3. WHO, UNDP/World Bank. WHO Special Programme for Research and Training in Tropical Diseases. Tropical Disease Research: progress 1975-94: highlights 1993-94: 12th Programme Report of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR)- Geneva 1995; p. 125-34.
4. Hayes R, Schofield C. Estimación de tasas de incidencia de infecciones y parasitosis crónicas a partir de la prevalencia: la enfermedad de Chagas en América Latina. Bol Ofic Sanit Panam 1990;108:308-16.

5. Oliveira J, Correa de Araujo R, Navarro M, Muccillo G. Cardiac thrombosis and thromboembolism in chronic Chagas' heart disease. Am J Cardiol 1983;52:147-51.
6. Arteaga Fernández E, Pereira Barretto AC, Ianni BM, Mady C, Lopes EA, Brito Vianna C, et al. Trombose cardiaca e embolia em pacientes fallecidos de cardiopatia chagasica cronica. Arq Bras Cardiol 1989;52:189-92.
7. Leonard R, Neville E, Hall R. Paradoxical embolism: a review of cases diagnosed during life. Eur Heart J 1982;3:362-70.
8. Carrasco Guerra H, Avilan L, Barboza J, Fuenmayor A, Dávila D, Pérez T. Enfermedad tromboembólica pulmonar y de los miembros inferiores en los pacientes con insuficiencia cardíaca congestiva global crónica. Arch Inst Cardiol Mex 1978;48:214-32.
9. Braga J, Labrunie A, Villaca F, do Nascimento E, Quijada L. Thromboembolism in chronic Chagas' heart disease. Rev Paul Med 1995;113:862-6.
10. Roldan Schiling V, Marin Ortuno F, Pineda Rocamora J, Climente Paya V, Martinez Martinez J, Marco Vera P, et al. Marcadores de hipercoagulabilidad y daño endotelial en pacientes con disfunción sistólica de origen isquémico. Rev Esp Cardiol 2001;54:1155-60.
11. Hagar JM, Rahimtoola SH. Chagas' heart disease. Curr Probl Cardiol 1995;20:825-924.
12. Lip G, Gibbs C. Does heart failure confer a hypercoagulable state? Virchow´s triad revisited. J Am Coll Cardiol 1999;33:1424-6.
13. Puigbo J, Giordano H, Suarez C, Acquatella H, Combellas I. Clinical aspects in Chagas' Disease. En: Madoery RJ, Madoery C, Camera MI, editores. Actualizaciones en la enfermedad de Chagas. Buenos Aires: Organismo oficial del Congreso Nacional de Medicina, 1992; p. 27-38.
14. Kistner R, Eklof B, Masuda E. Diagnosis of chronic venous disease of the lower extremities: the CEAP classification. Mayo Clinc Proc 1996;71:338-45.
15. Moleiro F, Rodríguez AE, Misticchio F, Ruesta V, Octavio JA, Álvarez E, et al. Utilidad de la aplicación de técnicas de modelado no lineal en el análisis de electrocardiogramas de pacientes con infección chagásica. Rev Esp Cardiol 2001;54: 1081-90.
16. Pujol del Poso A. Aplicaciones diagnósticas de los isótopos radiactivos. En: Series Monográficas Médicas. Medicina Nuclear. Barcelona: Editorial Científica Médica, 1980; p. 175-84.
17. The PIOPED Investigators. Value of the ventilation/perfusion scan in acute pulmonary embolism. Results of the Prospective Investigation of Pulmonary Embolism Diagnosis (PIOPED). JAMA 1990;263:2753-9.
18. Feigenbaum H. Masas intra cardíacas. En: Ecocardiografía. 5.ª ed. Buenos Aires: Editorial Médica Panamericana, 1994; p. 569-607.
19. Kistner R, Eklof B, Masuda E. Diagnosis of chronic venous disease of lower extremitis: the CEAP classification. Mayo Clin Proc 1996;71:338-45.
20. Roldan V, Marin F, Marco P, Climent V, Martinez JG, Monmeneu JV, et al. Anticoagulant therapy modifies fibrinolytic dysfunction in chronic atrial fibrillation. Haemostasis 2000;30:219-24.
21. Yamamoto K, Saito H. Diagnosis of predictive state of disseminated intravascular coagulation. Nippon Rinsho 1993;51:74-8.
22. Von Kaulla K, Von Kaulla E. Remarks on the euglobin lysis. En: Davidson J, Samana M, Desmoyers P, editors. Progress in chemical fibrinolysis and thrombolisis. New York: Daven Press, 1975; p. 131-49.
23. Espinoza R, Carrasco H, Belandria F, Fuenmayor A, Molina C, Martinez O. Life expectancy analysis in patients with Chagas' disease: prognosis after one decade (1973-1983). Intern J Cardiol 1985;8:45.
24. Rouvier J, Scazziota A. Factores y marcadores de riesgo de trombosis. Cuadernos de Trombosis. Buenos Aires: Editorial Infomed. Tomo I 1999; p. 39-40.
25. Fernández Pavón A, Martínez Brotons F. Estado actual de la profilaxis primaria de la enfermedad tromboembólica venosa. Valoración del riesgo y recomendaciones en las áreas médicas y quirúrgicas. Incidencia y factores de riesgo en la enfermedad tromboembólica venosa. Rev Iberoamer Tromb Hemostasia 1999; 12:49-55.
26. Haas S. European consensus statement on the prevention of venous thromboembolism. Blood Coagulation and Fibrinolysis 1993;4:55-8.
27. Alpert J, Dalen J. Epidemiology and natural history of venous thromboembolism. Progr Cardiovasc Dis 1994;36:417-22.
28. Prevention of Venous Thrombosis and Pulmonar Embolism. Consensus Conference. JAMA 1986;6:744-8.
29. Anderson F, Wheeler H. Physician practices in the management of venous thromboembolism. A community-wide survey. J Vasc Surg 1992;15:707-14.
30. Carter C. The natural history and epidemiology of venous thromboembolism. Prog Cardiovasc Dis 1994;36:423-38.
31. Coon W, Willis P. Recurrents of venous thromboembolism. Surgery 1973;73:823-7.