Importance of the dilution test in the dosage of coagulation factors XII and XI in plasma with positive lupus anticoagulant

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Summary

Introduction. Thrombosis is associated with acquired risk factors or hypercoagulable states. Antiphospholipid antibodies are found in infectious processes or associated with arterial or venous thrombosis. Among these is the lupus anticoagulant, which is considered an interference inhibitor because it prolongs phospholipid-dependent tests in vitro. **Objectives.** To relate the activity of factor XII and XI in patients with positive lupus anticoagulant. **Methodology.** Of 55 plasmas, 34 met the inclusion criteria. Factors XII and XI were dosed by metric clot methods. Samples less than 50 Ul/dl are considered low factor activity, to which the dilution test (parallelism) is performed. If a recovery greater than 15% of the factor is evidenced, it is considered interference. If, on the contrary, the result remains with little variation, it is confirmed. factor deficiency. **Results.** Of 34 dosages, 79.4% (27/34) presented a decrease in factor XI. A dilution test was performed, and 100% recovery of factor XI was observed; Regarding the dosage of factor XII, 29.4% (10/34) presented values below 50 IU/dl and 70.6% (24/34) normal, did not recover in 22.2% (2/9) presenting deficit. **Conclusions.** The study of a prolonged PTT is carried out due to suspicion of lupus anticoagulant or factor deficiency. The importance of the laboratory is that every time a decreased coagulation factor is found, plasma dilution must be done to determine if there is recovery of the factor or is a deficit of this.

Keywords: syndrome antiphospholipid, anticoagulant lupus, dilution, factor XII, factor XI

Introduction

Thrombosis is considered an alteration of multifactorial origin secondary to alterations in hemostasis, platelet function, leukocyte activation, and the vascular wall, which leads to clot formation and blood vessel obstruction, causing changes in blood flow. quality of life of people who suffer from it, such as paralysis, memory loss, and speech loss, among others. The disease can be caused by habits such as smoking, a sedentary lifestyle, dyslipidemia, obesity, or by diseases of genetic and/or acquired origin. The maintenance of the disease and the costs make it a public health problem.

Investigations carried out in experimental models have confirmed that plasmatic coagulation factors are also altered by different antiphospholipid antibodies. This type of antibody can recognize some epitopes of coagulation factors, evidencing the decrease in these proteins when functional activity is determined. As in vitro tests dependent on phospholipids, mainly PTT, are prolonged, the main factors to investigate are factors XII and XI because they are contact factors and normally when factor XII is decreased there are no clinical manifestations of bleeding, whereas when factor XII is decreased, there are no clinical manifestations of bleeding. XI is increased, and there is a hypercoagulable state. then the interference of these antibodies manages to demonstrate the decrease in the level of these factors. The important point to highlight is the role that the laboratory plays in being able to determine if there is indeed a decrease in the factors or if the decrease is due to the interference of the antibodies and that is where the plasma dilution test should be performed, making the dilutions depending on how low the factor is 1/2 or 1/4, 1/8 or 1/16 if there is recovery of the factors it indicates that it is due to interference of the antiphospholipid antibodies with the reagents used in the test. Among these antibodies, the most relevant are anticardiolipin antibodies, anti-phosphatidyl-serine antibodies, anti-Beta 2 glycoprotein antibodies, and lupus anticoagulants. Antibodies with persistent high titers are associated with arterial and venous thrombosis and recurrent miscarriages.

In the study, factors XII and XI were dosed because the variation in their activity may be an additional risk factor for thrombosis in patients with positive lupus anticoagulant.

Decreased factor XII concentration causes prolonged in vitro testing and may lead people to trigger thrombosis. This process is due to mutations in the F12 gene, located on the long arm of chromosome 5 (5 q 35.5), This gene codes for the protein factor XII or Hageman, and this factor is in the bloodstream in an inactive form until it is It encounters the walls of the injured vessels and is activated and initiates coagulation, interacting with factor XI. Factor XII also plays an important role in stimulating in-

flammation, a normal response of the body to infection, irritation, and other injuries. In addition, it interacts with another protein, prekallikrein, this interaction initiates chemical reactions that lead to the release of bradykinin that promotes inflammation, additionally, factor XII activates the fibrinolytic system, activating plasminogen to its active form plasmin, which destroys fibrin clots. and fibrinogen.

More than 20 mutations leading to factor XII deficiency have been identified, most of the mutations substitute amino acids in factor XII, altering its structure. The deficiency is inherited in an autosomal recessive pattern. This factor XII abnormality may predispose affected individuals to developing blood clots (thrombi) at an early age with a higher risk than the general population of developing deep vein thrombosis or bleeding disorders and unexplained repeated miscarriages in some affected women. Researchers are studying drugs to block (inhibit) factor XII as a potential therapy for people who are prone to developing blood clots.

Factor XI is encoded by the F11 gene, on the long arm of chromosome 4 (4q32-35), like many other coagulation factors, it is a serine protease. In humans, factor XI is activated by factor XIIa and thrombin, factor XIa activates factor IX by selective cleavage of peptide bonds. It is an autosomal recessive disorder; that leads to bleeding. Elevated levels of factor XI have been im-

plicated in cases of thrombosis generating a procoagulant state.

Antiphospholipid Syndrome (APS) is classified as an autoimmune disease with antibodies (Acs) isotype IgG / IgM/, IgA, it is characterized by presenting a clinical picture of arterial or venous thrombotic events and recurrent abortions, hemocytopenias, hemolytic anemias, neurological alterations with titers elevated and persistent antibodies. This antiphospholipid syndrome occurs in two forms: primary where no other autoimmune disease is evident and secondary where it is associated with an underlying autoimmune disease, the main one being Systemic Lupus Erythematosus. (1-4)

Antiphospholipid antibodies (aPL) are very heterogeneous immunoglobulins against cellular components or against coagulation factors. These antibodies act on both procoagulant and anticoagulant mechanisms at the membrane level of endothelial cells, platelets, and trophoblasts, among others, stimulating these cells to increase the expression and secretion of different molecules that favor pre-thrombotic states. (1-4)

In antiphospholipid syndrome, several pathophysiological mechanisms are proposed in which antiphospholipid antibodies alter the procoagulant and anticoagulant homeostatic reactions that occur on the cell membrane, blocking the binding of the protein with the negatively charged phospholipids, also blocking the ac-

cess of other proteins to these. phospholipids, therefore phospholipid-dependent coagulation reactions are inhibited, such as inhibition of protein C, protein S, Antithrombin (AT), Annexin 5 A, inhibits the anticoagulant activity of B2 GPI, alters the fibrinolytic system, decreases the PGI2 production, increased TXA2 in platelets, all these events are associated with pre- thrombotic states (5)

Elevated levels of coagulation factor XI predispose to thrombosis in patients with antiphospholipid syndrome, a relationship between altered factor XI activation and the syndrome has been discovered. Factor XI is a proenzyme that is activated to XIa by the action of activated factor XII or thrombin, factor XIa is responsible for the activation of factor IX, which leads to other enzymatic reactions for thrombin generation. (6-10)

There is evidence that the disulfide bonds in Factor XI are reduced to free thiols by the action of oxidoreductase enzymes on factor XI; The activation of factor XI by the action of thrombin or factor XIIa and treated with TRX1, significantly increased the reduced XIa, compared to the oxidized To verify the aforementioned, the ELISA test was performed to measure reduced factor XI in patients with antiphospholipid syndrome, it revealed that these patients have higher plasma levels of reduced FXI than normal controls, this contribu-

tes to understanding the predisposition to thrombosis when there are elevated levels of reduced FXI (11,12)

Positive lupus anticoagulants with anti B2GPI or anti- Cardiolipin are risk factors for thrombosis and for a pregnancy after 12 weeks. In a study carried out in people under 70 years of age with a first episode of thrombosis, it was shown that 3.1% of the people with deep vein thrombosis were positive for lupus anticoagulants. In a case study in women younger than 50 years, 17% of stroke patients were positive for lupus anticoagulant, and the risk was increased in women taking oral contraceptives. Lupus anticoagulant with the presence of aB2GPI correlates with a higher risk of thrombosis than lupus anticoagulant due to antithrombin autoantibodies, asymptomatic patients positive for lupus anticoagulant, antiphospholipid antibody and aB2GPI can be found with risk of a first event, they are called triple positives, these patients has high titers of antibodies, which bind to the LB major epitope in domain I of B2GPI; domain I aB2GPI, associated with a high risk of thrombosis due to the action of autoantibodies. Tests have also detected autoantibodies to the phosphatidylserine-prothrombin complex, this can help diagnose antiphospholipid syndrome and the association with high risk of thrombosis. On the other hand, in studies carried out, tissue factor (TF) could contribute to the prothrombotic state of patients

with persistent lupus anticoagulant and a history of thrombosis. (13,14)

Antiphospholipid antibodies require the presence of B2GP to bind to cardiolipin, aB2GPIs are of low affinity which is increased when bound to the B2GPI protein. Fibrinolysis is also affected due to the decrease in protein C, it alters the activity of thrombin-activated fibrinolysis inhibitors, as it is well known, this molecule is a plasminogen activator inhibitor, it indirectly prevents the clot from forming. degrade, on the other hand, the platelet is activated by the aB2GPI-B2GPI complex, increasing the production of TXA2, a powerful vasoconstrictor and platelet aggregator, it also inhibits Antithrombin that inhibits factor IXa , Xa and Thrombin. (fifteen).

The lupus anticoagulant depends on phospholipids, is responsible for prolonging coagulation time in vitro, and is associated with anticardiolipin antibodies, B2GPI, causing thrombosis and abortions. Oral direct thrombin and Xa anticoagulants, heparin, and vitamin K antagonists are used to prevent venous and arterial thrombosis but may be responsible for false-positive laboratory test results for lupus anticoagulants. (16)

The importance of factor XII is to participate in the activation of the intrinsic pathway of coagulation and plasminogen to generate plasmin. Deficiency of this factor is more associated with arterial and venous

thromboembolic complications leading to life-threatening myocardial infarction and pulmonary embolism, miscarriages, and cerebrovascular accidents, among others. (10)

The additional treatment of direct anticoagulants (DOACs) against the mentioned factors and to reduce aggregate thrombotic events in patients with APS refractory to conventional treatments who present nephropathy, should be administered with care, the anticoagulant action begins 2 hours after the first dose at Unlike vitamin K antagonist anticoagulants, if the anticoagulant is discontinued it rapidly loses its action, mostly eliminated in the urine (17-22).

The laboratory tests recommended by the subcommittee for scientific standardization for Lupus Anticoagulant (LAC) / antiphospholipid syndrome, are three lupus anticoagulants and anticardiolipin anticoagulant, aB2GPI, for a good diagnosis by the laboratory. IgG is more related to thrombosis than IgM, but in abortions it is IgM. Laboratory detection of lupus anticoagulants in anticoagulated patients is important because they can interfere with the result, causing false positives or negatives. For this, some reagents have heparin neutralizers capable of inhibiting unfractionated or low molecular weight heparin, and vitamin antagonists K may affect the detection of lupus anticoagulant. (23,24)

Taking into account that in some patients with Catastrophic antiphospholipid syn-

drome (CAPS), a rare and potentially fatal disease, is a systemic coagulopathy related to antiphospholipid antibodies that must be rapidly diagnosed and treated. It is characterized by clot formation in multiple organs high production of cytokines in a short time and high titers of antiphospholipid antibodies (25).

Among the recommendations for the management of antiphospholipid syndrome, the profile of high-risk antiphospholipid antibodies associated with thrombotic and obstetric antiphospholipid syndrome is important. Low-dose aspirin is recommended for asymptomatic carriers of antiphospholipid antibodies, patients with lupus erythematosus without thrombotic or obstetric antiphospholipid syndrome, and non-pregnant women with a history of obstetric antiphospholipid syndrome. Patients with antiphospholipid syndrome and first venous thrombosis should be treated with vitamin K antagonists with an INR 2-3. Rivaroxaban should not be used in patients with triple antiphospholipid antibody-positive antiphospholipid syndrome. (26)

Materials and methods

This research is descriptive, cross-sectional, correlational, and non-experimental. The project is endorsed by the Research Committee of the Universidad Colegio Mayor de Cundinamarca.

55 plasmas were selected, of which 34 plasmas met the inclusion criteria: samples from patients with prolonged TTP with positive lupus anticoagulant and exclusion criteria: samples from patients anticoagulated with direct inhibitors such as Rivaroxaban and Apixaban, direct factor X inhibitors and the inhibitor Dabigatran direct from thrombin

For the statistical analysis, a descriptive analysis of the continuous variables was carried out, the Shapiro-Wilk test was applied to define the type of distribution of the variables, in the variables of non-normal distribution the median, the Interquartile range (IR) and the maximum and minimum values. Proportions are presented in the categorical variables. For the bivariate analyses, the Pearson's Chi-square difference test, the Mann-Whitney test, the Kruskal-Wallis test, the Kruskal-Wallis test, and the binomial test were used.

A value of $p \leq 0.05$ was considered a significant difference. The statistical program SPSS 25 was used.

Coagulation factors XII and XI are measured in plasma by coagulometric methods using the CA 1500 and CS-2100i equipment. The test is performed by mixing plasma deficient in the factor to be quantified with the patient's plasma. A TTP is carried out for the case of factors XI and XII. The second data are interpolated into the specific calibration curve for each factor and the results are expressed in UI/dl.

The calibration curve is made with the dilutions defined for each factor, following the protocol of the programming manual of each equipment. The equipment automatically takes the coagulation times of each of the dilutions and graphs the curve with its corresponding factor activity.

Samples with results less than 50 Ul /dl are considered to have a low factor activity value, due to the possibility that it may be due to interference from the lupus anticoagulant, the parallelism test is performed, which was carried out by diluting the plasma ¼ and dosing the factor again. If a recovery of more than 15% of Factor is evident, it is considered that there is **recovery of the factor when diluting (parallelism).** If, on the other hand, the result of the factor remains with little variation in the different dilutions, that is, close to the initial value and is validated with the note: **No recovery of the factor is observed when performing the dilution (parallelism), which confirms factor deficiency.**

Results

Description of demographic characteristics with positive lupus anticoagulant

Of the total of 55 samples analyzed that were positive for lupus anticoagulant, 58.2% (32/55) had prolonged PTT and 3.6% (2/55) had normal PTT.

Table 1. Result of lupus anticoagulant.

		Negative	Positive	Total	p
Male	No.	11	18	29	0.968
	Percentage	52.4	52.9	52.7	
Female	No.	10	16	26	
	Percentage	47.6	47.1	47.3	
		21	3. 4	55	
p: Chi-squared difference					
There is no difference in the type of lupus anticoagulant according to sex					

Of the total of 34 samples positive for lupus anticoagulant, 18 (52.9%) corresponded to men and 16 (47.1%) corresponded to women. (Table 1)

Table 2. Relationship between age and gender

			Men	Women	Totals	P
Age	8 to 13 years	Number	8	1	9	NA
		Percentage	44.40%	6.30%	26.50%	
	14 to 23 years	Number	3	5	8	
		Percentage	16.70%	31.30%	23.50%	
	24 to 40 years	Number	4	6	10	
		Percentage	22.20%	37.50%	29.40%	
	> 40 years	Number	3	4	7	
		Percentage	16.70%	25.00%	20.60%	
	Total	Number	18	16	3. 4	0.552
P: Chi-squared difference						

There is no difference in the distribution of the type of lupus anticoagulant according to age groups.

It is noteworthy that in the ranges from 8 to 13 years, the number of men is greater than women, unlike the other ranges in which the values are very similar for both men and women.
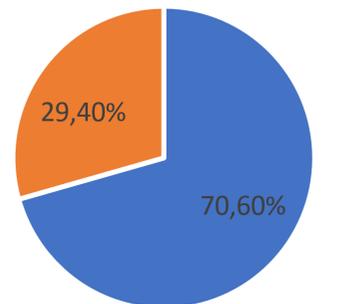
of 88.1. A normal value of 50 to 150 IU/ dL of factor XI is considered.
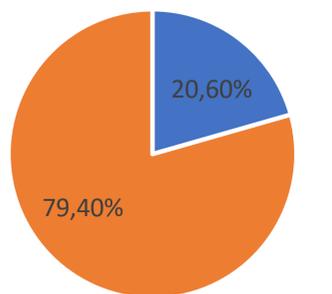
As can be seen, 20.6% (7/34) were normal and 79.4% (27/34) were below 50 IU /dl.

Graph 1. Factor XI according to UI/dl

The distribution of factor XI is not normal, median of 33.85 UI/ dL, interquartile range of 31.5, minimum of 33.2 and maximum

Graph 2. factor XII according to UI/dl

The factor XII distribution test is normal. Mean: 64.91. Standard deviation 29.19. Minimum: 4.5 Maximum 122.0. A normal range of 50 to 150 IU/dl is considered.

As can be seen, 29.4% (10/34) below 50 IU/ dl and 70.6% (24/34) normal.

Table 3. Relationship between Factor XI and Factor XII

			Factor XII		
			Average (50-150)	Abnormal	Total
Factor XI	Average (50-150)	Number	7	0	7
		% of the total	20.60%	0.00%	20.60%
	Abnormal	Count	17	10	27
		% of the total	50.00%	29.40%	79.40%
	Total	Count	24	10	3. 4
		% of the total	70.60%	29.40%	100.00%

Of the total the 34 samples, 20.6% were found with both factors between normal limits, and 29.4%, both with abnormal factors. The factor with the greatest alteration is factor XI.

Parallelism, ¼ dilution for factor XI

Of the 27 samples with factor XI, less than 50 IU/dl, parallelism (¼ dilution) was made to 20, which corresponds to 74% (20/27).

Table 4. Frequency of Factor XI recovery with 1/4 dilution

	No	%
He recovered	20	100
Total	20	100

Of the 20 samples of factor XI to which parallelism was performed, 100% recovered

Of the 10 samples with factor XII less than 50 IU/ dL, parallelism was performed (¼ dilution) to 9, corresponding to 90% (2/9), not recovered.

Table 5. Frequency of Factor XII recovery with 1/4 dilution

	No	%
I do not recover	2	22.2
He recovered	7	77.8
Total	9	100

Of the nine samples to which parallelism was performed, 22.2% (2/9) did not recover, which suggests factor deficiency and 77.8% (7/9) did recover.

Of the 10 samples to which parallelism was performed for factor XI and XII, 7 recovered in both factors.

Discussion

The results of this study of factors XII and All samples with positive AL were dosed with Factor XI and Factor interference of antiphospholipid acs in 79.4%, in which 100% recovery of the factor was observed. The data observed in the samples analyzed reflect the importance that in all hemosta-

sis laboratories where these factor dosage tests are performed and these are decreased, dilution tests are performed to evaluate interference by possible antibodies, and in this way the results issued are used by doctors to follow the best clinical conduct for the benefit of patients. As recommended by the WFH guidelines (27)

It should be noted that when determining factor XII of the samples positive for lupus anticoagulant, interference from antiphospholipid antibodies was found in 29.4%, for which reason the dilution test (1/4) was performed, in which recovery was observed in 77.8% and 22.2% did not recover, confirming the factor deficiency. These data make clear the importance of carrying out the dilution in hemostasis laboratories, since samples with real factor XII deficiency can be found, but also others that are simply caused by the interference of antiphospholipid antibodies. When we receive samples with prolonged PTT we can find ourselves in different scenarios: samples with real deficiency of some coagulation factor (Factor XI and XII), samples with positive lupus anticoagulant accompanied by a deficiency of some contact factor, or only samples with lupus anticoagulant positive with normal factor dosage after performing dilution tests.

This experience with this work makes clear the importance of the clinical laboratory in carrying out these tests and that the responsible professionals can carry out the

complementary studies that are required to give a correct and clear interpretation of the results. Well-performed and interpreted tests will facilitate clinical correlation and thus the treating physician will adopt the best behaviors, especially since these patients will receive chronic anticoagulation. On the other hand, it must be remembered that the laboratory must always have excellent communication with the treating physician to confirm any finding of interest. As recommended by the WFH guidelines (27)

When carrying out the comparative study between factors XII and XI, it was found that 29.4% of the two factors were altered, there is a higher incidence of alteration in factor XI, as well as a higher frequency in male children.

A notable finding is that the longest PTT values were found in children from 8 to 13 years of age compared to the other age groups.

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Conflict of interests

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