4. ARTÍCULOS PUBLICADOS

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Clinical Study and Follow-Up of 100 Patients With the Antiphospholipid Syndrome

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Objectives: **To study the clinical characteristics at diagnosis and during follow-up of patients with the antiphospholipid syndrome (APS) and to analyze the influence of treatment on their outcome.**

Patients: **One hundred patients with APS were included (86% female and 14% male; mean age, 36 years). Sixty-two percent had primary APS and 38% had APS associated with systemic lupus erythematosus (SLE). The median length of follow-up was 49 months.**

Results: **Fifty-three percent of the patients had thromboses, 52% had thrombocytopenia, and 60% of the women had pregnancy losses. Patients with APS associated with SLE had a higher prevalence of hemolytic anemia ($P = .02$), thrombocytopenia (platelet count lower than 100×10^9/L) ($P = .004$), antinuclear antibodies ($P = .0002$), and low complement levels. Fifty-three percent of the patients with thrombosis had recurrent episodes (86% in the same site as the previous thrombotic event). Recurrences were observed in 19% of the episodes treated with long-term oral anticoagulation, in 42% treated prophylactically with aspirin, and in 91% in which anticoagulant/antiaggregant treatment was discontinued ($P = .0007$). Multivariate analysis showed that prophylactic treatment and older age had an independent predictive value for rethrombosis. Prophylactic treatment during pregnancy (usually with aspirin) increased the live birth rate from 38% to 72% ($P = .0002$).**

Conclusions: **Patients with APS have a high risk of recurrent thromboses. Long-term oral anticoagulation seems to be the best prophylactic treatment to prevent recurrences. Prophylactic treatment with aspirin during pregnancy reduced the rate of miscarriages remarkably.**

INDEX WORDS: Antiphospholipid syndrome; thrombosis; obstetric complications; prophylactic treatment.

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THE ANTIPHOSPHOLIPID SYNDROME (APS) is an autoimmune disorder characterized by recurrent arterial and venous thromboses, fetal losses, or thrombocytopenia in association with the presence of antiphospholipid antibodies (aPL), that is, anticardiolipin antibodies (aCL) or lupus anticoagulant (LA) (1). This syndrome is called "primary" (2), if not associated with other underlying disease, or "secondary," if it appears in association with other autoimmune disorders, mainly systemic lupus erythematosus (SLE) (3,4).

The aPL are a heterogeneous family of immunoglobulins directed against complexes of phospholipid and plasma proteins, the latter being considered as cofactors. The best known of these cofactors are beta-2-glycoprotein I and prothrombin (5). The aPL associated with clinical manifestations of the APS usually show dependence of these protein cofactors (6). Despite the advances experienced over the past several years in understanding the immunologic characteristics of aPL, the etiopathogenesis of the APS is not clear. However, there is increasing knowledge of the clinical picture and the management of the disease. Only a few studies have analyzed the long-term follow-up of patients with APS. The present study analyzes the clinical characteristics at the beginning and during the follow-up of 100 patients with APS in a single center and evaluates the influence of anticoagulant/antiaggregant treatment on the outcome of these patients.

PATIENTS AND METHODS

Patients

We retrospectively studied 100 patients diagnosed and followed-up in our center of APS between 1985 and 1998. These were the first 100 patients seen during that time, excluding those without any follow-up data. The mean age was 36 years (range, 13-79) with 86 (86%) females and 14 (14%) males. Sixty-two (62%) patients had primary APS, and 38 (38%) had APS associated with SLE. The median length of follow-up from the time of diagnosis was 49 months (range, 4-150). Forty-one patients had a follow-up period longer than 5 years.

Methods

The patients' clinical records were carefully reviewed according to a preestablished protocol. APS diagnosis was made according to the criteria proposed by Harris et al (7). SLE diagnosis was based on the revised criteria reported by the American College of Rheumatology (8). Diagnosis of deep venous thrombosis was based on clinical grounds and confirmed by Doppler ultrasonographic scans or venography. Pulmonary embolism was diagnosed by ventilation-perfusion lung scanning or pulmonary angiography. Cerebrovascular ischemic episodes were diagnosed by the appropriate clinical picture and computed tomographic scanning or magnetic resonance imaging. Angina or myocardial infarction were diagnosed according to the clinical presentation, electrocardiographic changes and elevated levels of cardiac enzymes. Endocardial valve disease was diagnosed by echocardiography. Thromboses at other sites were diagnosed by angiographic studies, and only the episodes confirmed by imaging techniques were accepted. Because of the study design, we could not influence treatment decisions. In most cases, oral anticoagulation was chosen to treat venous thromboses, and antiaggregants were selected for the first epidose of arterial thrombosis, mainly cerebrovascular ischemia, but oral anticoagulation was given if arterial rethrombosis occurred.

Thrombocytopenia was considered when the platelet count was less than 150×10^9/L on a minimum of two occasions (mild, if platelet count was between 100 and 150×10^9/L; moderate, if between 50 and 100×10^9/L; or severe, if less than 50×10^9/L). Hemolytic anemia was diagnosed by a positive Coombs' test and laboratory data showing active hemolysis. APL were found in all patients on two occasions at least. ACL were measured by a standardized enzyme-linked immunosorbent assay (ELISA) (9), and the LA was assessed by coagulation assays following the criteria of the Subcommittee for the Standardization of Lupus Anticoagulants of the International Society of Thrombosis and Hemostasis (10). Antinuclear antibodies (ANA) were determined by indirect immunofluorescence using mouse liver as substrate. Anti-dsDNA were determined by Farr's ammonium sulphate precipitation technique. Anti-Ro (SSA), anti-La (SSB), anti-Sm, and anti-RNP antibodies were investigated by counterimmunoelectrophoresis using calf and rabbit thymus and human spleen extracts. Complement components (C3 and C4) were quantitated by radial immunodiffusion and CH50 by Lachmann's hemolytic technique.

Statistical Analysis

Results are shown as mean ± SD and 95% confidence intervals (CI) or ranges are reported. Chi-square or Fisher's exact tests were used for comparing qualitative variables and ANOVA or *t*-tests for quantitative variables. To study recurrences, the follow-up time for each patient with thrombosis was divided into periods that began with the last thrombotic episode and ended with either recurrence or the end of the follow-up. This was then referred to as episodes. Kaplan-Meier curves of thrombosis-free probability were plotted, and log-rank and Breslow-Gehan tests were used for comparisons using the trend version when indicated. These tests were selected because Breslow-Gehan gives greater weight to early observations and the log-rank test gives equal value to all observations and is then more sensitive to later events. Multivariate analysis was performed using the proportional hazard model with covariates. In this model, withdrawal of oral anticoagulants was introduced when indicated as a time-dependent covariable. Relative risk (RR) was taken as the ratio of the risk of rethrombosis per unit time for a patient with a given set of high-risk variables to the risk for a patient without these high-risk variables. The statistical analysis was performed using the SPSS-PC 6.0 and BMDP-PC statistical packages for Windows.

RESULTS

General Characteristics

The median time from the first feature attributable to APS until diagnosis was 21 months (range, 1-516). Table 1 shows the events leading to diagnosis of the APS. During the follow-up, 53 (53%) patients developed thrombosis, 52 (60%) women had spontaneous pregnancy losses, and 52 (52%) patients developed thrombocytopenia. Among the latter, thrombocytopenia was mild in 13 (25%) patients, moderate in 24 (46%), and severe in 15 (29%). Table 2 shows the cumulative clinical manifestations; because our hospital is a referral center for the APS, mainly for obstetric complications, the number of women with miscarriages likely was higher than other reports. Table 3 shows the immunologic profiles at diagnosis. During the follow-up, only one patient with primary APS evolved into SLE, and four (4%) patients died: one of pulmonary embolism, one with multi-infarct dementia due to aspiration pneumonia, one of infectious complications during a flare up of SLE, and one of acute cardiac failure caused by mitral prosthetic valve thrombosis.

Table 1: Events Leading to the Diagnosis of the Antiphospholipid Syndrome

Clinical Event	%
Obstetric complications	34
Deep venous thrombosis in legs and/or pulmonary embolism	19
Cerebral ischemia	16
Thrombocytopenia and/or hemolytic anemia	15
Ischemic cardiomyopathy	2
Ocular disease (optic neuropathy or ocular vaso-occlusive disease)	5
Arterial ischemia in legs	2
Non-infectious endocarditis	1
Renal thrombotic microangiopathy	1
Splenic infarction	1
Portal thrombosis	1
Hepatic nodular regenerative hyperplasia	1
Prolonged aPTT	1
SLE flare up	1

Abbreviations: aPTT, activated partial thromboplastin time; SLE, systemic lupus erythematosus.

Comparison Between "Primary" and "Secondary" APS

Patients with APS associated with SLE had a higher prevalence of hemolytic anemia (24% *v* 7%; $P = .02$), moderate or severe thrombocytopenia (platelet count lower than 100×10^9/L) (58% *v* 27%; $P = .004$), ANA (97% *v* 67%; $P = .0002$), low C3 level (35% *v* 11%; $P = .012$), low C4 level

Table 2: Cumulative Clinical Manifestations of Patients with the APS

Clinical Manifestation	n = 100	%
Miscarriage	52/86	60
Thrombocytopenia	52/100	52
Arterial thrombosis	32/100	32
Venous thrombosis	25/100	25
Valvular heart disease	20/100	20
Autoimmune hemolytic anemia	13/100	13
Migraine headaches	13/100	13
Livedo reticularis	12/100	12
Epilepsy	3/100	3
Dementia	2/100	2
Chorea	1/100	1

Table 3: Immunologic Characteristics of Patients With the APS

	n° Patients (Positive/Tested)	%
LA	69/100	69
aCL (IgG)	74/100	74
Low	11/74	15
Moderate	11/74	15
High	52/74	70
aCL (IgM)	32/100	32
Low	10/32	31
Moderate	7/32	22
High	15/32	47
BFP-STS	12/52	23
ANA	75/95	79
Anti-DNA	31/87	36
C3 low	19/91	21
C4 low	26/91	29
CH50 low	34/91	37

Abbreviations: LA, lupus anticoagulant; aCL, anticardiolipin antibodies; BFP-STS, biologic false-positive serologic test for syphilis; ANA, antinuclear antibodies; anti-DNA, anti-DNA native antibodies.

(46% *v* 17%; $P = .005$) and low CH50 (57% *v* 24%; $P = .003$). When the ANA titer was analyzed, we found a titer prevalence greater than 1/200 in APS higher with SLE ($P = .01$).

Thrombotic Events

Fifty-three patients (41 women and 12 men) had one or more episodes of thrombosis: 21 (40%) patients had venous thromboses, 28 (53%) arterial thromboses and four (7%) both arterial and venous thromboses. The presence of LA at diagnosis was statistically associated with thrombotic events (62% *v* 32%, $P = .005$). Twenty-five (47%) patients experienced one thrombotic episode (11 with venous thromboses and 14 with arterial thromboses), and 28 (53%) had recurrent events. Nineteen (68%) patients had only one recurrence, and 9 (32%) had two or more. Recurrences occurred in the same region in 24 (86%) patients (10 in the venous and 14 in the arterial circulation).

Ninety-six thrombotic events were observed in the above-referred 53 patients and 43 episodes were recurrent (26 arterial and 17 venous). The median time to recurrence was 71 months, and the probability of remaining free of thrombosis 5 years after a previous thrombotic event was 52% (CI 40-64%) (Fig 1). When we studied the influence of the different treatment modalities on thrombotic recurrence, we observed seven (19%) recurrent events (median, 96 months; probability of remaining free of thrombosis at 5 years, 81%; CI 65-97%) in patients prophylactically treated with oral anticoagulant therapy, 15 (42%) recurrences (median 75 months, probability of remaining free of thrombosis at 5 years 58%; CI, 38-78%) among patients prophylactically treated with aspirin, and 21 (91%) recurrences (median 48 months, probability of remaining free of thrombosis at 5 years 22%; CI, 5% to 39%) among the patients in which prophylac-

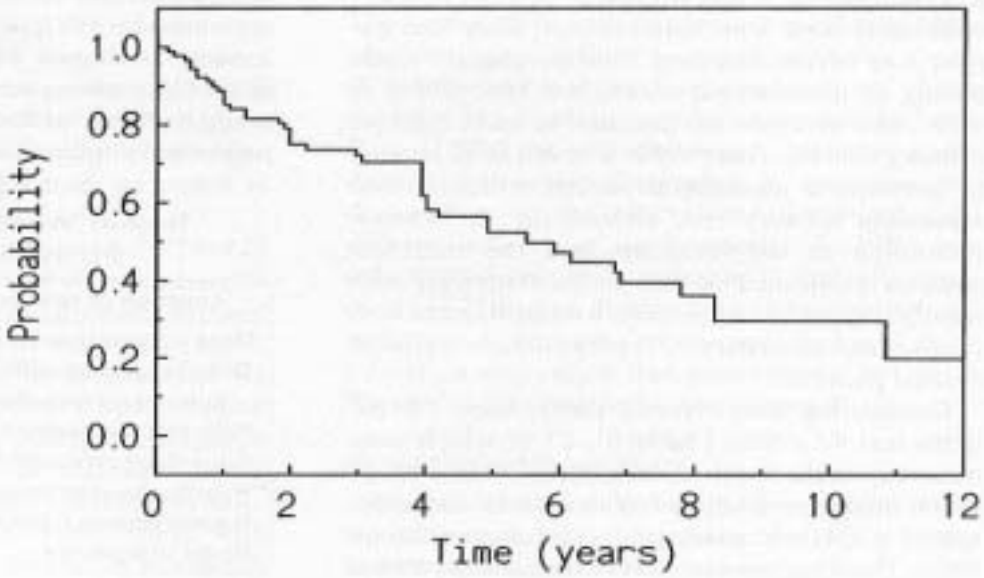

Fig 1. Actuarial probability of remaining free of recurrent thrombosis for the 96 episodes.

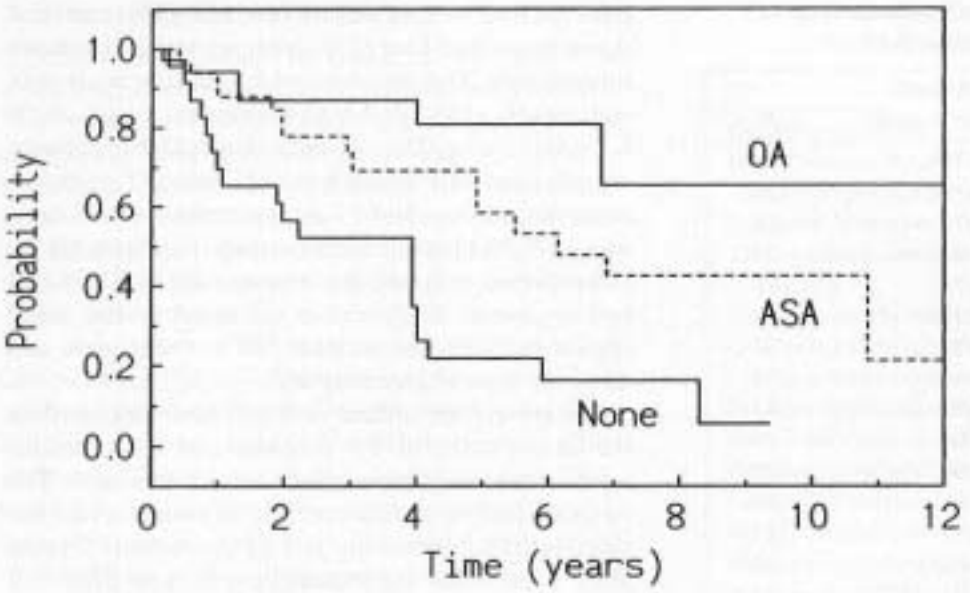

Fig 2. Actuarial probability of remaining free of recurrent thrombosis comparing the different treatment modalities: long-term oral anticoagulation (OA), aspirin (ASA), and no treatment (None). Comparison by Breslow-Gehan and log-rank tests show statistically significant differences among them ($P = .0007$ and $P = .0001$, respectively).

tic (anticoagulant or antiaggregant) treatment was withdrawn (Breslow-Gehan, $P = .0007$; log-rank, $P = .0001$) (Fig 2). Oral anticoagulant treatment was withdrawn in 21 instances, and this was changed to aspirin six times. Fifteen recurrences were observed (median, 9 months) after withdrawal of oral anticoagulation treatment, and withdrawal, considered as a time-dependent variable, was significantly related to rethrombosis ($P = .0001$). In the multivariate analysis, both the type of prophylactic treatment ($P = .0001$; RR no treatment = 1.0, RR aspirin = 0.37, RR oral anticoagulants = 0.23) and older age ($P = .02$; RR age > 50 years = 2.24) were associated with rethrombosis. Only one patient had severe bleeding (retroperitoneal) while having an international normalized ratio (INR) of 4.32. The number of patients in both groups, primary and secondary APS, was not large enough to perform a meaningful statistical analysis to separately identify risk factors for thrombosis. According to the literature, and the statistical analysis performed in our series (although with insufficient statistical power), we assume that both primary and secondary APS patients have a similar clinical picture.

Considering only venous thromboses, 25 patients had 42 events (Table 4), 17 of which were recurrent. In the multivariate analysis, both the type of prophylactic treatment ($P = .0004$) and older age ($P = .04$) were associated with venous rethrombosis. Three recurrences were observed in patients undergoing anticoagulation treatment: two patients had an episode of deep venous thrombosis while on coumadin (the INR was 1.80 in one and 2.46 in the other), and one developed a recurrence of deep venous thrombosis in the legs during the postpartum period while on subcutaneous low-molecular-weight heparin.

Thirty-two patients had 54 arterial thromboses (Table 5), 26 of which were recurrent. Again the type of treatment ($P = .02$) and older age ($P = .05$) influenced rethrombosis. During anticoagulant therapy, only four recurrences were observed: one patient had renal thrombotic microangiopathy while on coumadin (INR within a range of 2.5 to 3.5); another developed a thrombus on a mitral prosthetic valve when coumadin treatment was changed to intravenous unfractionated heparin; the third patient had thrombus on a biological prosthetic valve

Table 4: Venous Thrombotic Events in Patients With the APS

Location of venous thrombosis	n (42)	%
Deep venous thrombosis in legs	32	77
Deep venous thrombosis in legs and pulmonary embolism	3	7
Pulmonary embolism	2	5
Superficial venous thrombosis	2	5
Subclavian vein thrombosis	1	2
Retinal venous thrombosis	1	2
Portal thrombosis	1	2

Table 5: Arterial Thrombotic Events of Patients With the APS

Manifestation	n (54)	%
Stroke	23	42
Myocardial infarction or angina	8	15
Non-infectious endocarditis or valve thrombus	3	6
Retinal arterial thrombosis or amaurosis fugax	4	7
Arterial thrombosis in legs	5	9
Renal thrombotic microangiopathy	3	6
Ischemic optic neuropathy	2	4
Splenic infarction	1	2
Transverse myelitis	1	2
Several arterial sites simultaneously	4	7

while on coumadin with an INR within a range of 2.5 to 3.5; and the last had a stroke while the INR was 1.80.

Obstetric Complications

Pregnancy losses occurred in 103 out of 169 (61%) pregnancies before the diagnosis of APS. Most (72%) miscarriages occurred during the first trimester. After the diagnosis of APS, 43 subsequent pregnancies occurred in 29 women. Prophylactic treatment with low-dose aspirin (100 mg/d) was administered in 36 of these pregnancies under the following regimens: aspirin alone in 18 pregnancies, aspirin with prednisone (because of SLE manifestations) in 12, aspirin with subcutaneous low-molecular-weight heparin in 5, and aspirin with low-molecular-weight heparin, prednisone, and immunoglobulins (due to previous treatment failure) in 1. Prednisone alone was administered in two pregnancies and low-molecular-weight heparin alone in one. Four women declined pharmacological treatment, and all their pregnancies ended in miscarriage.

Treatment led to a live birth rate of 72% (28 pregnancies). However, the rate of obstetric complications, such as preeclampsia, prematurity, and intrauterine growth retardation, was high (36%). Considering the total number of pregnancies, we observed a live birth rate of 38% in women without treatment (66 out of 173 pregnancies) compared with 72% in those who received treatment (28 out of 39 pregnancies) ($P = .00002$). When low-dose aspirin treatment, alone or in combination with other drugs, was considered, the rate of live births was 75% in comparison to 38% in women without treatment ($P < .0001$). More specifically, the rate of live births was 78% (14 of 18 pregnancies) in pregnancies treated with low-dose aspirin alone, 83% (10 of 12 pregnancies) in those treated with aspirin plus prednisone, and 60% (3 of 5 pregnancies) in those treated with aspirin plus low-molecular-weight heparin. The only pregnancy treated with aspirin, low-molecular-weight heparin, prednisone, and immunoglobulins ended in miscarriage. One of the two pregnancies treated with prednisone alone and the one in which only low-molecular-weight heparin was administered also ended in miscarriage. However, because aspirin was used as first-line treatment for these pregnancies in our protocols, and low-molecular-weight heparin was considered as a second step, a selection of high-risk cases can be expected in the low-molecular-weight heparin regimens. Moreover, comparison between low-dose aspirin alone and other regimens was not possible because of the small number of patients in each group. The above analysis was made on the entire series; when primary and secondary APS were analyzed separately, the proportion of pregnancy losses and the prophylactic treatment received were similar.

Endocardial Valve Disease

Twenty (20%) patients developed heart valve lesions. Mitral valve disease was found most commonly (80% of patients): valve thickening in 3 patients, mitral regurgitation in 10, and noninfectious vegetations (Libman-Sacks endocarditis) in 4. The aortic valve was the second most frequently involved valve (40% of patients): valve thickening in one patient and dysfunction in seven (aortic regurgitation in all and additional stenosis in four). Two (10%) patients developed tricuspid valve disease with valve thickening in one case and dysfunction in the other case. Among the 20 patients with valve involvement, 14 (70%) had only 1 valve involved (mitral in 11 patients, aortic in 3), 5 (25%) had disease of 2 valves (mitral and aortic in 4 patients, mitral and tricuspid in 1 patient), and 1 patient had simultaneous lesions in three valves (mitral, aortic and tricuspid).

Hematologic Abnormalities

Apart from thrombocytopenia (present in 52% of the patients as previously described), 13 (13%) patients developed hemolytic anemia.

Other Clinical Manifestations

Other clinical features associated with aPL were livedo reticularis (12% of patients), migraine headache (13%), epilepsy (3%), multi-infarct dementia (2%), and chorea (1%).

DISCUSSION

We observed that primary and secondary APS have a similar clinical spectrum. Only moderate to severe thrombocytopenia, hemolytic anemia, ANA and low levels of complement were significantly more frequent in patients with SLE. In these patients, a relationship has been reported between aPL and thrombocytopenia (4), but other factors, such as antiplatelet antibodies, seem to be involved (11,12), accounting for the higher prevalence of thrombocytopenia. Several studies have demonstrated an association between hemolytic anemia and the IgM isotype of aCL in SLE (4,13). Moreover, immunoglobulins eluted from red blood cells of patients with SLE and hemolytic anemia have aCL activity (14), suggesting a possible role in the development of active hemolysis. Nevertheless, the antigenic specificity of antibodies to red blood cells detected by Coombs' test in SLE is variable and hemolytic anemia may occur without aPL. The finding of ANA is one criteria for the classification of SLE (8), and their greater frequency in "secondary" APS is expected. Among patients with positive ANA, the titer was higher in secondary than in primary APS. Piette et al (15) postulated that an ANA titer higher than 1/320 should be an exclusion criteria for primary APS, and this finding may indicate progression to SLE. In our series, only one patient with primary APS evolved into SLE during follow-up. Finally, low levels of complement are associated with lupus activity (16) and not with the classical manifestations of APS, which are mainly due to thrombosis secondary to a hypercoagulable state. Vasculitis with immunoglobulin and complement deposition is not implicated (17,18).

Thrombotic events are the clinical hallmark of the APS, occur in the venous and arterial circulations and have a high recurrence rate (19). Shah et al (20) reported that the 29% of patients with a previous diagnosis of the APS developed further thrombotic episodes during 10 years of follow-up. Rosove et al (21) found a recurrence rate of 53% after the first thrombotic event with most recurrences (91%) in the same region. Krnic-Barrie et al (22) described a recurrence rate of 44%, while Khamashta et al (23) observed recurrences in 69% of patients. In our series, 55% of patients developed recurrent thrombotic episodes. In most patients (86%), they occurred in the same region, a peculiar pattern reported previously (21,23,24). Factors responsible for this pattern are unknown. In our series, deep venous thromboses in the legs was the most common event in the venous circulation (77%), and cerebral ischemic attacks were most frequent in the arterial circulation (42%). Similar findings recently have been reported (20-23). Over the past years, several studies have tried to identify a subset of patients with APS having a higher risk for recurrent thrombosis, who may benefit from long-term anticoagulant or antiaggregant treatment. The presence of LA seems to be the strongest risk factor for thrombosis in patients with primary APS and SLE (25,26), as confirmed in our study.

Concerning management, we identified long-term oral anticoagulation as the best prophylactic treatment for recurrent thrombosis. This treatment was more effective than antiaggregants or no treatment. When oral anticoagulation was administered, the recurrence rate was 19%, as compared with 42% for those treated with aspirin and 91% for those in which treatment was discontinued. Moreover, the 5-year probability of developing a recurrence was less with oral anticoagulation, and they occurred later. Khamashta et al (23) noted that thrombosis recurrence was higher in the first 6 months after withdrawal of anticoagulants with a median time of 2 months. In our study, the median time to recurrence after coumadin withdrawal was 9 months. Thus, after the cessation of anticoagulation, recurrences quickly develop, suggesting that patients with the APS should receive long-term therapy after the first event.

Some authors have reported that high-intensity anticoagulation (INR $>$ 3) was more effective than low-intensity anticoagulation (INR $<$ 3) in preventing recurrences, although the bleeding rate was higher (21,23). However, the recommended therapeutic INR range remains controversial, because aPL, particularly LA, may interfere with the prothrombin time determination, rendering the INR system invalid for patients with APS (27,28). Robert et al (29) reported that the INR system may be valid for monitoring anticoagulant treatment, although LA interfer with some reagents, leading to an overestimation of the effect of oral anticoagulation. In our protocols, we tried to achieve an INR ranging between 2.5 and 3.5. Only seven recurrences and one major bleeding were observed. At

the time of the recurrent thrombotic event, three patients had an INR less than 2.5; in two cases, there was an additional factor, such as the postpartum period or the change from oral anticoagulation to intravenous unfractionated heparin. In the two remaining cases, thromboses recurred whereas the INR was within the preestablished range. We suggest that patients with a strong LA demonstrating potential interference with prothrombin time be identified and that the INR should be between 2.5 and 3.5, using reagents with no LA interference.

Recurrent miscarriage, the other clinical hallmark of the APS (1,2), was present in 60% of the women in our study. Although fetal loss in the third trimester is more characteristic of APS (30), and is frequently associated with placental infarcts and vascular thrombosis (31), early pregnancy loss is more common (32,33). In our series, 68% of miscarriages occurred in the first trimester; the immunopathology is not well known because placental infarcts are infrequent. Other mechanisms have been suggested, such as impairment of embryonic implantation (34), a fall in cytokine levels (mainly interleukin-3) (35) involved in fetal implantation and development, and disturbances in human placental choriogonadotropin secretion (36). In our study, 62% of pregnancies without treatment ended in miscarriage. Some previous studies reported that pregnancy loss was higher than 70% without treatment (32,33,37). When prophylactic treatment was administered, the live birth rate was 72%, similar to other reports (32,37). These data highlight the need for women with aPL to receive appropriate information and prophylactic treatment when pregnant.

With respect to the best prophylaxis, we observed that low-dose aspirin (100 mg/d) was effective and safe when administered throughout pregnancy. Aspirin, alone or in combination with other drugs, led to a live birth rate of 75%. These results are better than two recent prospective controlled studies comparing low-dose aspirin with aspirin plus heparin (38,39), in which the rate of live births rose from 42% and 44% to 71% and 80% (38,39) when heparin was added to low-dose aspirin. However, these differences could be due to the characteristics of the population selected, because the women in these studies had at least three consecutive spontaneous pregnancy losses. This resulted in the selection of a subset of women with APS with the highest risk for new pregnancy loss. Our study included women with a single miscarriage, or women without obstetric complications in whom APS was diagnosed on the basis of other clinical manifestations, but who received prophylactic treatment for the first pregnancy. Despite the good results achieved with prophylaxis, fetal complications such as intrauterine growth retardation, prematurity and preeclampsia are not uncommon (37). Thirty-six percent of women who delivered a viable infant in our study had obstetric complications. Rai (38) and Kutteh (39) found 24% and 15%, respectively, of obstetric complications in successful pregnancies, although differences were not observed between women treated with aspirin and aspirin plus heparin. Therefore, pregnancy in women with APS should be considered high risk and closely monitored.

In conclusion, patients with APS have a high risk of recurrent thrombosis and long-term oral anticoagulation seems to be the best prophylactic treatment. Multicenter prospective trials are needed to determine the optimal range of anticoagulation without increasing the bleeding risk. Prophylactic treatment during pregnancy markedly reduces the rate of miscarriages and, in our experience, low-dose aspirin is adequate treatment.

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RESUMEN

Objetivo: Estudiar las características clínicas en el momento del diagnóstico y durante el seguimiento de los pacientes con síndrome antifosfolipídico y analizar la influencia del tratamiento sobre su curso evolutivo.

Material y métodos: Para ello se estudiaron 100 pacientes con el diagnóstico de síndrome antifosfolipídico (86% mujeres y 14% hombres con una edad media de 36 años). El 62% de los pacientes estaba diagnosticado de un síndrome antifosfolipídico primario y el resto tenía asociado un lupus eritematoso sistémico. La mediana del tiempo de seguimiento fue de 49 meses.

Resultados: Las principales manifestaciones clínicas del síndrome antifosfolipídico fueron trombosis en el 53% de los pacientes, trombocitopenia en el 52% y abortos o pérdidas fetales en el 60% de las mujeres. Los pacientes con síndrome antifosfolipídico asociado al lupus eritematoso sistémico se diferenciaron de la forma primaria por presentar una mayor prevalencia de anemia hemolítica (p=0,02), trombocitopenia con un recuento de plaquetas inferior a $100x10^9$/L (p=0,004), anticuerpos antinucleares (p=0,0002) y niveles más bajos del complemento. En el 53% de los pacientes las trombosis fueron recurrentes. Las recurrencias ocurrieron habitualmente en el mismo territorio vascular que el episodio previo (86%). La mediana del tiempo hasta la recurrencia fue de 71 meses y la probabilidad de permanecer libre de recurrencia de trombosis a los 5 años después de un episodio trombótico previo fue del 52%. Respecto al tratamiento administrado, un 19% de los episodios

tratados con anticoagulación indefinida presentó recurrencias respecto a un 42% de los episodios trombóticos tratados con antiagregación y un 91% de los episodios en los que el tratamiento anticoagulante o antiagregante fue interrumpido permaneciendo sin profilaxis (p=0,0007). El análisis multivariante puso de manifiesto que los factores predictores independientes de retrombosis fueron el tratamiento profiláctico y la edad avanzada. Cuando se analizaron de forma independiente las trombosis arteriales y venosas se obtuvieron los mismos resultados en ambas. Respecto a las complicaciones obstétricas, la administración de un tratamiento profiláctico durante el embarazo (generalmente ácido acetilsalicílico a dosis de 100 mg/día) incrementó de forma significativa la proporción de los embarazos que finalizaron con un recién nacido vivo (38% sin tratamiento profiláctico respecto a 72% con profilaxis; p=0,0002), aunque el porcentaje de complicaciones tales como retraso en el crecimiento intrauterino, prematuridad o preeclampsia fue elevado (36%).

Conclusiones:

1. Los pacientes con síndrome antifosfolipídico con antecedentes de trombosis tienen un alto riesgo de recurrencia.
2. El mejor tratamiento profiláctico es la administración de anticoagulantes orales de forma indefinida.
3. El tratamiento profiláctico durante el embarazo reduce de forma considerable el riesgo de abortos o pérdidas fetales, aunque la incidencia de complicaciones obstétricas es elevada.

4.2. Prevalence and clinical significance of antiprothrombin antibodies in patients with systemic lupus erythematosus or with primary antiphospholipid syndrome. Haematologica 2000; 85: 632-637.

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Prevalence and clinical significance of antiprothrombin antibodies in patients with systemic lupus erythematosus or with primary antiphospholipid syndrome

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ABSTRACT

Background and Objectives. **Antibodies to prothrombin (all) have been identified in patients with antiphospholipid antibodies, but their clinical significance is not well known. The aim of our study was to investigate their prevalence and association with clinical manifestations of the antiphospholipid syndrome (APS) in patients with primary APS or with systemic lupus erythematosus (SLE).**

Design and Methods. **A series of 177 patients with autoimmune diseases was studied: 70 with primary APS and 107 with systemic lupus erythematosus. A control group of 87 healthy volunteers were included in the study. all were investigated in sera by an ELISA, using human prothrombin as antigen fixed in irradiated polystyrene plates.**

Results. **all prevalence in patients with autoimmune disease was 47% (57% and 40% in patients with primary APS or with SLE, respectively) significantly higher than in controls (5%) ($p<0.0001$). In the whole series, thrombotic events were more prevalent in patients with all (45% vs 28%; $p=0.02$). Moreover, all was found to be an independent risk factor for arterial thrombosis (OR=2.4; $p=0.04$). Similarly, in patients with SLE, all were associated with both arterial and venous thrombosis (35% vs 14%; $p=0.01$), although only IgG-all (OR=3.7; $p=0.01$) had an independent value as risk factor for thrombosis. However, a relationship between all and thrombosis was not found in primary APS. all were associated with thrombocytopenia only in patients with primary APS (OR=6.7; $p=0.007$).**

Interpretation and Conclusions. **all seem to be a serological marker of prothrombosis in autoimmune diseases, mainly in SLE patients and/or in the arterial territory.**

Key words: antiprothrombin antibodies, antiphospholipid syndrome, systemic lupus erythematosus, thrombosis, miscarriage, thrombocytopenia

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The association between clinical features of recurrent thrombosis, miscarriage and thrombocytopenia, and antiphospholipid antibodies (aPL) is named the antiphospholipid syndrome (APS).[1] There is now general agreement that antiphospholipid antibodies in autoimmune diseases are not directed towards phospholipids alone, but to complexes of phospholipids and proteins that are bound to these phospholipids and act as cofactors.[2] In this way, anticardiolipin antibodies (aCL) need the β_2-glycoprotein I to bind to cardiolipin[2] and lupus anticoagulants (LA) need β_2-glycoprotein I and/or prothrombin to recognize phospholipids.[3,4] Recently, antibodies to β_2-glycoprotein I and to prothrombin have also been identified in patients with aPL, using these molecules as antigens fixed in the absence of phospholipids in irradiated polystyrene plates.[5,6] Moreover, anti-β_2-glycoprotein I antibodies (aβ_2GPI) seem to be more specific for thrombosis in patients with APS and systemic lupus erythematosus (SLE) than aCL (5). aβ_2GPI, and antiprothrombin antibodies (all) are frequently found in sera of patients with LA[6] but, so far, available data about the clinical associations and usefulness of all are controversial.[7-11] It was reported that some non-neutralizing all associated with LA could cause hypoprothrombinemia and abnormal bleeding by increased clearance of prothrombin-antiprothrombin antibody complexes from the circulation.[7] However, the majority of all detected in sera of patients with antiphospholipid antibodies are low-affinity antibodies and do not cause hypoprothrombinemia or bleeding.[8] In addition, an association between all and thrombosis has been suggested.[9-11] The aim of the present work was to study the clinical significance of all in a large series of consecutive and unselected patients with primary APS or SLE, and to determine whether these autoantibodies could help us to identify a subset of patients with an increased risk of clinical manifestations.

Design and Methods

Patients

We studied 177 patients with autoimmune diseases [153 females and 24 males, mean age 38 years

(range 15-79)]: 70 with primary APS and 107 with SLE. The diagnosis of APS was made according to previously described criteria.[12] The diagnosis of SLE was based on the revised criteria reported by the American College of Rheumatology.[13] The control group was formed of 87 healthy volunteers (73 females and 14 males) without autoimmune disease, bleeding disorders, thrombosis or history of pregnancy losses.

Methods

Blood samples were drawn into trisodium citrate and in non-anticoagulated tubes (Becton Dickinson, Rutherford, NJ, USA). Platelet-free plasma was obtained by double centrifugation [first at 2,000 g (10 min, 22°C) and then at 5,000 g (10 min, 4°C)], frozen and stored at –70°C.

Detection of anti-prothrombin antibodies

Microtiter plates Maxisorp (Nunc, Roskilde, Denmark) were coated with 80 µL/well of 10 µg/mL human prothrombin (Diagnostica Stago, Asnières, France) in phosphate buffered saline, pH 7.4 (PBS) overnight at 4°C. After washing with PBS containing 0.1% Tween 20 (Merk, Munchen, Germany), wells were blocked (1 h, 22°C) with 150 µL of 1% bovine serum albumin (BSA) (Sigma, St. Louis, MO, USA) in PBS-Tween. After washing, wells were then incubated (1 h, 22°C) with 100 µL of samples diluted (1:100) in PBS-Tween-1% BSA. After new washing, plates were incubated (1 h, 22°C) with 100 µL of horseradish peroxidase-conjugated anti-human IgG (1:2,500) and IgM (1:1,500) (Dako, Glostrup, Denmark) in PBS-Tween-1% BSA. Finally, for color developing, 100 µL of 0.04% (0.4 mg/mL) orthophenylenediamine dihydrochloride (Sigma) diluted in phosphate-citrate buffer (pH=5.0) containing 25 µL /100 mL H_2O_2 (Sigma) were added. After incubation (10 min, 22°C), the reaction was stopped with 25 µL of 2N H_2SO_4, and optical density was measured at 492 nm (OD_{492}). Intra and interassay coefficients of variation were 4.56 and 13.3 for IgG, and 4.85 and 9.4 for IgM. In each assay, one positive serum for all and 8 negative sera were used as controls. OD_{492} values higher than 5 standard deviations (SD) above the mean of negative controls were considered positives: low positive between 5 and 7 SD, moderate positive between 7 and 9 SD, and high positive above 9 SD.

Detection of anti-cardiolipin antibodies

The aCL were measured by a commercially available ELISA (Cheshire Diagnostics, Chester, UK).

Detection of lupus anticoagulant

LA was assessed by coagulation assays using activated partial thromboplastin time, diluted Russell's viper venom time and tissue thromboplastin inhibition. Tests were also performed in mixtures with normal plasmas or phospholipids following the criteria of the *Subcommittee for the Standardization of Lupus Anticoagulants of the International Society of Thrombosis and Hemostasis*.[14]

Detection of anti-β_2-glycoprotein I antibodies

The aβ_2GPI were measured using an ELISA.[5] Briefly, microtiter plates (Maxisorp, Nunc, Roskide, Denmark) were coated (16 h, 4°C) with 10 µg/mL purified human β_2-glycoprotein I (Stago, Asnières, France) in carbonate buffer (pH=9.6). After washing, coated plates were blocked (1 h, 22°C) with 5% BSA (Sigma) in PBS-Tween, and then incubated (1 h, 22°C) with samples diluted in PBS-Tween plus 1% BSA. After new washing, plates were incubated (1 h, 22°C) with horseradish peroxidase-conjugated anti-human IgG and anti-human IgM (Dako) diluted in PBS-Tween-BSA. For color developing, ortho-phenylenediamine dihydrochloride (Sigma) diluted in phosphate citrate buffer (pH=5.0) plus H_2O_2 was added, incubated (10 min, 22°C) and the OD_{492} read. In each assay, 10 negative sera were used as controls and OD_{492} values higher than 5 SD above the mean of them were considered positive: low positive between 5 and 7 SD, moderate positive between 7 and 9 SD, and high positive above 9 SD.

Plasminogen activity

Plasminogen activity was determined using a specific chromogenic assay (Chromogenix, Molndal, Sweden). Plasminogen present in the plasmas was converted to an active plasminogen-streptokinase complex by an excess of streptokinase. Then, plasminogen-streptokinase complex catalyzed the splitting of *p*-nitroaniline (pNA) from the substrate H-D-Val-Leu-Lys-pNA*2HCl (S-2251) and the pNA release was measured at 405 nm.

Statistical analysis

To study the relationship between autoantibodies and clinical manifestations (thrombosis, fetal miscarriage and thrombocytopenia) the whole series of patients with autoimmune diseases (SLE and primary APS) (n=177) was analyzed. Taken into account that SLE and primary APS are different autoimmune diseases with their own diagnostic criteria, in a second phase we studied patients with SLE and with primary APS separately, and the analysis was repeated considering, first, patients with SLE (n=107), and, second, patients with primary APS (n=70). Results are shown as mean±SD. Chi-square or Fisher's exact tests were used for comparing qualitative variables and ANOVA and the t-test for qualitative ones. A $p<0.05$ was considered statistically significant. Multivariate analysis using a backward stepwise logistic regression was performed and odds ratios (OR) reported. The statistical analyses were performed using the SPSS-PC 6.0 statistical packages for Windows.

Results

Clinical characteristics

Clinical manifestations of patients with APS are shown in Table 1. Primary and secondary APS had a similar clinical spectrum, although thrombocytopenia (platelet count lower than 100×10^9/L) was more frequent in APS associated with SLE (51% vs 30%; $p=0.02$).

Prevalence of antiprothrombin antibodies

all were positive in 5% (4/87) of healthy volunteers. The prevalence of all was 47% in all patients with

Table 1. Clinical manifestations of patients with antiphospholipid syndrome.

	Primary APS (70) N(%)	Secondary APS (43) N(%)	Total (113) N(%)
Miscarriage	31 (60)	24 (60)	55 (60)
Thrombosis	39 (56)	21 (49)	60 (53)
Arterial	22 (31)	11 (26)	33 (29)
Venous	19 (27)	13 (30)	32 (28)
Thrombocytopenia <100x10⁹/L	21 (30)	22 (51)*	43 (38)

*p<0.02. Difference between primary and secondary APS. APS: antiphospholipid syndrome.

Table 2. Prevalence of different antiphospholipid antibodies.

	Autoimmune disease (177) N(%)	Primary APS (70) N(%)	SLE (107) N(%)
LA	77 (44)	44 (63)	33 (31)
aCL	85 (48)	52 (74)	33 (31)
IgG	71 (40)	44 (63)	27 (25)
IgM	26 (15)	14 (20)	12 (11)
all	83 (47)	40 (57)	43 (40)
IgG	58 (33)	27 (39)	31 (29)
IgM	41 (23)	22 (31)	19 (18)
a*β₂GPI	60 (34)	32 (46)	28 (26)
IgG	36 (20)	18 (26)	18 (17)
IgM	44 (25)	25 (36)	19 (18)

APS: antiphospholipid syndrome; SLE: systemic lupus erythematosus LA: lupus anticoagulant; aCL: anticardiolipin antibodies; all: antiprothrombin antibodies; aβ₂GPI: anti-β₂-glycoprotein I antibodies; APS: antiphospholipid syndrome; SLE: systemic lupus erythematosus.

autoimmune disease, and 57% and 40% respectively in patients with primary APS and SLE with and without classical aPL. The IgG class of all was the most frequent isotype. Table 2 shows the prevalences of the different aPL. A total of 27 patients (15.3%) had all the autoantibodies (aCL, LA, aβ₂GPI, and all), and 51 patients (28.8%) had only one antibody (12 aCL, 10 LA, 8 aβ₂GPI, and 21 all). When we analyzed the correlation between the different aPL in the whole population with autoimmune disease (n=177), we found a close relationship between all and classic aPL (LA and aCL), preferably between autoantibodies of the same isotype. Specifically, 62% and 64% of patients with LA and aCL, respectively, were all positive. A good correlation between all and aβ₂GPI was also observed. Tables 3 and 4 show the relationship among these autoantibodies. A total of 81% (62/77) of patients with LA had all and/or aβ₂GPI.

Table 3. Relationship between antiprothrombin antibodies and other antiphospholipid antibodies in patients with autoimmune disease (N=177).

	LA (77) N (%)	aCL (85) N (%)	IgG-aCL (71) N (%)	IgM-aCL (26) N (%)	aβ₂GPI (60) N (%)	IgG-aβ₂GPI (35) N (%)	IgM-aβ₂GPI (44) N (%)
all	48 (62)°	54 (64)°	47 (66)°	17 (65)°	35 (58)°	22 (61)°	28 (64)°
IgG	37 (48)°	37 (44)*	36 (51)°	7 (27)	22 (37)	17 (47)°	16 (36)
IgM	21 (27)	30 (35)°	23 (32)°	11 (42)°	22 (37)*	12 (33)	20 (46)°

*p<0.05; *p<0.01; °p<0.001. LA: lupus anticoagulant; aCL: anticardiolipin antibodies; aβ₂GPI: anti-β₂-glycoprotein I antibodies; all: antiprothrombin antibodies.

Relationship with thrombotic events.

In patients with autoimmune disease, a significant association between all and thrombosis was found, as shown in Table 4. This association was seen in the IgG isotype, but not in the IgM. The association with thrombosis was also seen for LA, aCL, IgG-aCL and IgG-aβ₂GPI, although LA was the only variable included in the best model for thrombosis in the multivariate analysis (OR=6.4; $p<0.0001$). For arterial

Table 4. Relationship between antiprothrombin antibodies and thrombosis in different subsets of patients.

	Thrombosis arterial	Thrombosis venous	Thrombosis total
Autoimmune disease (n=177)	N=34	N=34	N=63
all:			
Positive (83)	24 (29%)**	17 (21%)	37 (45%)*
Negative (94)	10 (11%)	17 (18%)	26 (28%)
IgG-all:			
Positive (58)	17 (29%)*	16 (28%)*	29 (50%)*
Negative (119)	17 (14%)	18 (15%)	34 (29%)
IgM-all:			
Positive (41)	10 (24%)	5 (12%)	14 (34%)
Negative (136)	24 (18%)	29 (21%)	49 (36%)
Primary APS (n=70)	N=22	N=19	N=39
all :			
Positive (40)	15 (38%)	9 (23%)	22 (55%)
Negative (30)	7 (23%)	10 (33%)	17 (57%)
IgG-all:			
Positive (27)	9 (33%)	8 (30%)	15 (56%)
Negative (43)	13 (30%)	11 (26%)	24 (56%)
IgM-all:			
Positive (22)	9 (41%)	3 (14%)	11 (50%)
Negative (48)	13 (27%)	16 (33%)	28 (58%)
SLE (n=107)	N=12	N=15	N=24
all			
Positive (43)	9 (21%)*	8 (19%)	15 (35%)*
Negative (64)	8 (26%)*	7 (11%)	9 (14%)
IgG-all:			
Positive (31)	3 (5%)	8 (26%)*	14 (45%)*
Negative (76)	4 (5%)	7 (9%)	10 (13%)
IgM-all:			
Positive (19)	1 (5%)	2 (11%)	3 (16%)
Negative (88)	11 (13%)	13 (15%)	21 (24%)

*p<0.05; *p<0.01; °p<0.001. all: antiprothrombin antibodies; APS: antiphospholipid syndrome; SLE: systemic lupus erythematosus.

thrombosis, we observed a significantly higher prevalence of events in patients with aII and IgG-aII. This relationship was also found for LA, aCL, IgG-aCL, aβ2GPI, IgG-aβ2GPI, and IgM-aβ2GPI, in the univariate analysis. In the multivariate analysis, aII added a significant independent value (OR=2.4; p=0.04) to LA (OR=5.9; p=0.0001) in the best model for arterial thrombosis. For venous thrombosis, a higher prevalence was found in patients with positive IgG-aII, LA and IgG-aCL, although only LA was included (OR=3.4; p=0.002) in the best model.

In the SLE group, aII were significantly associated with thrombosis, as shown in Table 4. Only IgG-aII isotype but not IgM showed significant association. Moreover, the prevalence of thrombosis in patients with LA, IgG-aCL or with IgG-aβ2GPI was significantly higher than patients who tested negative. IgG-aII (OR=3.7; p=0.01) added an independent predictive value to LA (OR=8.0; p=0.0001) in the multivariate analysis. When arterial thromboses were analysed, a strong association with aII and its IgG isotype was seen. Furthermore, patients with LA (30% vs 3%; p=0.0001) or with IgG-aβ2GPI (33% vs 7%; p=0.004) developed these thrombotic events more frequently than patients without these autoantibodies. Regarding venous thrombosis, only IgG-aII and LA (30% vs 7%; p=0.002) showed a significant association. It was not possible to perform a multivariate analysis in the subsets of patients because the number of events was not large enough to have adequate statistical power.

In the primary APS group, no association was found between aII and total thrombosis (Table 4). Only IgG-aCL was associated with thrombosis (66% vs 39%; p=0.02). When we studied arterial and venous thrombosis separately, a significant relationship was found only between aCL (39% vs 11%; p=0.02) and their IgG isotype (41% vs 15%; p=0.02) and arterial thrombosis.

Relationship with miscarriage

In the autoimmune disease group constituted by 153 women, miscarriages occurred more frequently in women with positive aII than in women who tested negative, although this difference did not reach statistical significance (43% vs 35%; p=0.2). Only aCL appeared to be significantly associated with miscarriages (49% vs 31%; p=0.02).

In the group of 101 women with SLE, no association with aII (28% vs 28%; p=0.9) was observed. Miscarriages occurred in 42% of women with positive LA compared to 21% with negative LA (p=0.03). Moreover, they were significantly more frequent in women with positive aCL than with negative aCL (43% vs 21%; p=0.02). The multivariate analysis included aCL in the best predictive model (OR=2.9; p=0.02).

In the group of 52 women with primary APS, no significant association between aPL and miscarriage was found. Sixty-three percent of women with positive aII had miscarriages compared to 55% of those who tested negative (p=0.5).

Relationship with thrombocytopenia

In the autoimmune disease group, thrombocytopenia (<100x10⁹/L) was not significantly associated with aII. Only LA (42% vs 16%; p=0.0001) and IgG-aβ2GPI (42% vs 23%; p=0.03) emerged as being associated with thrombocytopenia.

Thrombocytopenia was detected in 25% of patients with SLE, but it was not significantly associated with aII. Only LA was statistically associated with thrombocytopenia (42% vs 18%; p=0.007).

In the primary APS group, thrombocytopenia was significantly associated with aII (45% vs 10%; p=0.0009) and their two isotypes, IgG (44% vs 21%; p=0.03) and IgM (50% vs 21%; p=0.01). Also, it was associated with LA (41% vs 12%; p=0.006), aCL (37% vs 11%; p=0.03) and IgG-aβ2GPI (56% vs 21%; p=0.007). The multivariate analysis showed aPT (OR=6.7; p=0.007) and IgG-aβ2GPI (OR=4.0; p=0.02) as the only two independent predictor factors of thrombocytopenia.

Plasminogen activity

All samples had a normal plaminogen activity with a mean of 109±17%. Differences between groups were not observed, and no relationship with thrombotic events or with aII was found.

Discussion

The clinical relevance of aII has not yet been established and available data are controversial. Some of them, but not all, show LA activity,[15,16] suggesting that these autoantibodies are heterogeneous. Our results, showing that aII are detected in 62% of samples with LA, and, taken together, aII and aβ2GPI are found in 81% of samples with LA, suggest that the ELISA methods used in the present work cannot identify all the samples with LA activity, and, consistently, could not replace the detection of LA in the study of aPL.

In patients without autoimmune disease, Palosuo *et al.*[9] found a close relationship between aII levels and deep venous thrombosis and pulmonary embolism in middle-aged men. Vaarala *et al.*[10] also found that aII imply a risk of myocardial infarction in middle-aged men. Recently, Bertolaccini *et al.*[11] found aII in 28% of patients with SLE, and the presence of aII correlated with a history of thrombosis: 53% of patients with aII had thromboses compared to 32% of those without aII. Contrariwise, Eschwège *et al.*[17] did not find a relationship between aII and a history of venous thrombosis in unselected patients; aII could be demonstrated in only 4% of the 122 plasmas tested, which is a similar prevalence to that found by us in the group of healthy people. Our results showed that aII were associated with arterial thromboses in the patients with autoimmune diseases and with both arterial and venous thromboses in patients with SLE. Moreover, IgG isotype but not IgM was useful as a thrombosis marker, unlike the results obtained by Bertolaccini *et al.*,[11] who found an important correlation between the presence of both isotypes and vascular events. However, in agreement with previous reports,[18] LA seems to have the strongest predictive value for thrombosis. Moreover, Horbach *et al.*[19] also showed that LA correlates best with both arterial and venous thromboses in patients with SLE, and neither aII nor aβ2GPI gives additional information about a thrombotic risk. Pengo *et al.*[20] reported an aII prevalence of 50% in patients with

aCL and thrombosis but no association with thrombosis was found. Forastiero *et al.*[21] observed that aβ2GPI, but not all, give an increased risk for venous thrombosis in patients with aPL. Finally, Swadzba *et al.*[22] reported no correlation between all and thrombosis in patients with SLE. The differences between the above mentioned works can be explained by the different selection criteria of their populations, the number patients'proved, and technical variations in all ELISA. all are more frequently detected in ELISA when prothrombin is bound to phosphatidylserine-coated ELISA plates using calcium ions than when prothrombin is bound to γ-irradiated or high-activated PVC plates.[15] Furthermore, we should not forget that many works are retrospective and they analyze the relationship between the presence of an autoantibody, such as all or aβ2GPI, in a unique sample of patients with a history of thrombosis. Therefore, prospective studies in large series of patients are needed to determine the risk that subjects carrying all and other autoantibodies have of developing thrombosis. According to our results, all could be a serologic marker of thrombosis mainly in patients with SLE.

The role of all in the pathogenesis of thrombosis is unknown. It was suggested that all showing LA activity cause prolongation of clotting tests, mainly the kaolin clotting time, by inhibiting both the prothrombinase and tenase complexes.[23] However, this coagulation profile seems to confer a weak risk of thrombosis, in contrast with the dilute Russell's viper venom time, which selectively evaluates prothrombin conversion and is prolonged in the presence of aβ2GPI.[24] The all seem to increase the affinity of prothrombin for negatively charged phospholipids competing with the binding of other coagulation factors for them and inhibiting the conversion of prothrombin into thrombin and, thus, probably hampering protein C activation.[23] However, it has been recently suggested that all are unable to inhibit protein C activity.[25] More recently, Puurunen *et al.*[26] found that all crossreact with plasminogen in patients developing myocardial infarction. These crossreactive all can interfer with fibrinolytic function of plasminogen and favor the development of thrombosis. However, our results do not support this hypothesis, because we found a normal plaminogen activity in all samples and no relationship was found with all.

The relationship between all and miscarriage is still less clear. We did not find an association between all and fetal losses either in primary APS or in SLE patients, similarly to results reported by Forastiero *et al.*[21] and Falcon *et al.*[27] in women with aPL and by Bertolaccini *et al.*[11] in women with SLE. However, Ailus *et al.*[28] showed that increased levels of all were associated with secondary abortions in women who had already delivered at least one live child. These discreparicies are probably due to differences in baseline characteristics of the samples of studied patients. It has been suggested that vascular thrombosis and pregnancy loss are due to the reduction of surface-bound annexin V by aPL,[29] leading to a hypercoagulable state in the placenta and vascular endothelium. Binding of aPL to vascular endothelium can be mediated by phospholipid-binding proteins, such as β2-glycoprotein I[30] and other cofactors, although whether all is involved remains uncertain.

Finally, we observed an association between all and thrombocytopenia in patients with primary APS but not in those with SLE, in agreement with previous reports.[11,22] Although it can not be excluded that all could bind to platelets and cause thrombocytopenia, it seems that the presence of antibodies directed to specific platelet glycoproteins could be the main mechanism of thrombocytopenia, similar to the situation in idiopathic thrombocytopenic purpura.[31]

In conclusion, all seem to be a serologic marker of thrombosis in autoimmune diseases, particularly in patients with SLE. Prospective studies are needed to confirm these results.

Potential implications for clinical practice

1. all are a serologic marker of thrombosis in autoimmune diseases, particularly in patients with SLE. These autoantibodies could, therefore, be useful together with the classical aPL for evaluating the potential risk of thrombosis.
2. all are not associated with other manifestations of primary or secondary APS, such as miscarriage or thrombocytopenia.

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FJMR, JCR, JF and DT designed the study, interpreted the results and wrote the paper. FJMR, JCR, DT and GE performed the laboratory studies. FJMR, RC, JF, GE and JCR recruited patients and controls and were the clinicians responsible for the clinical management of the patients and clinical data acquisition. FC and JB took care of the pregnant patients and were responsible for the obstetrical program and data. JB, AO and MI are the heads of their respective departments and gave final aproval for the manuscript submission. All the authors critically revised the paper. The order of authors was decided on the basis of the size of their contribution.

Disclosures

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RESUMEN

Introducción: Los anticuerpos dirigidos contra la protrombina se han identificado en pacientes con anticuerpos antifosfolipídicos, aunque su significado clínico es todavía incierto.

Objetivo: Investigar la prevalencia de los anticuerpos antiprotrombina en pacientes con síndrome antifosfolipídico primario o lupus eritematoso sistémico y su posible asociación con las principales manifestaciones clínicas asociadas a los anticuerpos antifosfolipídicos.

Material y métodos: Se estudiaron 177 pacientes con una edad media de 38 años (153 mujeres y 24 hombres): 70 tenían un síndrome antifosfolipídico primario y 107 un lupus eritematoso sistémico. Se incluyó un grupo control formado por 87 voluntarios sanos. Para investigar la presencia de anticuerpos antiprotrombina en el suero de los pacientes se utilizó un ELISA en la que se usaba protrombina purificada humana como antígeno, que se fijaba a los pocillos de las placas de poliestireno previamente irradiadas. Los coeficientes de variación intraensayo fueron 4,56 para el isotipo IgG y 4,85 para el IgM. Los coeficientes de variación interensayo fueron respectivamente 13,3 y 9,4 para los isotipos IgG e IgM.

Resultados: La prevalencia de los anticuerpos antiprotrombina en la población estudiada de pacientes con enfermedades autoinmunes fue del 47% (57% en el síndrome antifosfolipídico primario y 40% en el lupus eritematoso sistémico), significativamente superior a la obtenida en el grupo control (5%) ($p<0,0001$). El

isotipo IgG fue el más frecuente. En la población de enfermedades autoinmunes la prevalencia de trombosis fue superior en los pacientes con anticuerpos antiprotrombina (45% vs 28%; p=0,02), aunque esta asociación sólo se observó con el isotipo IgG (50% vs 29%; p<0,01). Cuando se realizó un análisis multivariante en el que se incluyeron, además de los anticuerpos antiprotrombina, la presencia de anticoagulante lúpico, anticuerpos anticardiolipina o anticuerpos anti-η2 glicoproteína I, se obtuvo que los anticuerpos antiprotrombina eran un factor de riesgo independiente para trombosis arteriales (OR=2,4; p=0,04) junto con el anticoagulante lúpico. De forma similar, la prevalencia de trombosis en pacientes con lupus eritematoso sistémico fue superior en aquellos que tenían anticuerpos antiprotrombina (35% vs 14%; p=0,01). Esta asociación se observó sólo con el isotipo IgG (45% vs 13%; p<0,001. El análisis multivariante identificó el isotipo IgG de los anticuerpos antiprotrombina como un factor de riesgo independiente para trombosis (OR=3,7; p=0,01) junto con el anticoagulante lúpico. En el grupo de pacientes con síndrome antifosfolipídico primario no se observó asociación entre trombosis y anticuerpos antiprotrombina. Respecto a las complicaciones obstétricas, no se demostró asociación entre abortos o pérdidas fetales y anticuerpos antiprotrombina en mujeres con lupus eritematoso sistémico o con síndrome antifosfolipídico primario. Finalmente, los anticuerpos antiprotrombina se asociaron de forma significativa con trombocitopenia (recuento de plaquetas

<100x10^9/L), aunque sólo en pacientes con síndrome antifosfolipídico primario (OR=6,7; p=0,007).

Conclusiones:

1. Los anticuerpos antiprotrombina son muy prevalentes en pacientes con síndrome antifosfolipídico primario o con lupus eritematoso sistémico.
2. En pacientes con lupus eritematoso sistémico el isotipo IgG de los anticuerpos antiprotrombina constituye un factor de riesgo independiente para el desarrollo de trombosis, tanto arteriales como venosas.

4.3. Clinical significance of acquired activated protein C resistance in patients with systemic lupus erythematosus. Lupus 2002; 11 :730-735.

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PAPER

Clinical significance of acquired activated protein C resistance in patients with systemic lupus erythematosus

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Antiphospholipid antibodies (aPL) may induce acquired activated protein C resistance (acquired APCR). The role of acquired APCR in patients with systemic lupus erythematosus (SLE) is not well known. To evaluate the prevalence of acquired APCR and its association with clinical manifestations we studied 103 consecutive SLE patients and 103 matched controls. APCR in the undiluted test and after dilution in factor V deficient plasma, factor V Leiden, protein C and S, lupus anticoagulant, and anti-cardiolipin, anti-β2-glycoprotein I and anti-prothrombin antibodies were determined. Factor V Leiden was found in 4% in both patients and controls. The prevalence of acquired APCR was 22% for the undiluted assay and 17% in the diluted test. In SLE patients, acquired APCR was associated with aPL (39 *vs* 13% in undiluted assay, $P = 0.007$; and 33 *vs* 7% in the diluted test, $P = 0.001$). Arterial thromboses were found in 24% of patients with acquired APCR and in 6% of patients without ($P = 0.04$). However, no relationship was found with venous thrombosis. Acquired APCR was also associated with pregnancy losses: miscarriages in 70% of women with acquired APCR *vs* 32% in those without ($P = 0.03$). Thus, in SLE patients acquired APCR seems to be associated with increased prevalence of arterial thrombosis and pregnancy losses. *Lupus* (2002) **11**, 730–735.

Key words: acquired activated protein C resistance factor V Leiden; antiphospholipid antibodies; systemic lupus erythematosus; thrombosis

Introduction

Thrombosis related to antiphospholipid antibodies (aPL) is a common clinical manifestation of patients with systemic lupus erythematosus (SLE),[1] but the mechanisms of these thrombosis are not well known. Several mechanisms have been described including the interference of aPL in the protein C anticoagulant system[2] by the inhibition of the protein C activation by thrombomodulin,[3] the decrease of free protein S levels,[4,5] or the impairment of the catalytic activity of activated protein C preventing activated factor V (Va) degradation.[6–8] Activated protein C resistance (APCR) was first described in 1993,[9] found to be associated with venous thrombosis,[10] and in 1994 it was demonstrated to be due to a single point mutation in the factor V gene causing a factor V molecule (factor V Leiden) resistant to activated protein C inactivation.[11] Later, an acquired form of APCR was described associated with pregnancy,[12] the use of oral contraceptives[13] or in patients with aPL.[14] However, if acquired APCR represents a thrombotic mechanism in such patients is not well known.

Our objectives were to evaluate the prevalence of acquired APCR in patients with SLE and to investigate its possible relationship with aPL and with aPL-associated clinical manifestations.

Patients and methods

Patients

We studied 103 consecutive patients diagnosed of SLE according to the criteria of American College of Rheumatology.[15] They were 97 females and six

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males with a median age of 38 years (range from 15 to 72 years). In addition 103 normal volunteers (age- and sex-matched with SLE patients) were studied as the control group. Informed consent was obtained from all the participants.

Methods

Blood samples were drawn from a clean antecubital venipuncture without venocclusion in the morning with the patient sitting and resting. Samples for coagulation studies were obtained in tubes containing 3.8% trisodium citrate (1:9, vol:vol, Becton Dickinson, Rutherford, NJ, USA) and platelet-free plasma was immediately obtained by double centrifugation: first at 2000*g* for 10 min at 22°C, and then at 5000*g* for 10 min at 4°C. Plasma was immediately aliquoted and stored at −70°C. For factor V Leiden determination samples were drawn in tripotassium EDTA tubes (Becton Dickinson) and 100 µl of whole blood were transferred into tubes containing lysis buffer (5 mol/l guanidine thiocyanate, 1.3% (w/v) Triton X-100, and 50 mmol/l Tris–HCl, pH 6.4), and frozen at −70°C. Sera for autoantibody studies were drawn in tubes containing no anticoagulants (Becton Dickinson).

Detection of lupus anticoagulant (LA)

LA was assessed by coagulation assays following the criteria of the Subcommittee for the Standardization of Lupus Anticoagulants of the International Society of Thrombosis and Hemostasis.[16] The kaolin clotting time (KCT) was performed according to Exner *et al.*[17] and the dilutued Russell's viper venom time (dRVVT) according to Thiagarajan *et al.*[18] In each patient positive for lupus anticoagulant, the LA profile was evaluated by comparing the ratios obtained in KCT and in dRVVT tests according to Galli *et al.*;[19] when the ratio of the KCT exceeded that of the dRVVT, the patient was considered to have a KCT-LA profile and, inversely, when the ratio of the dRVVT exceeded that of the KCT, the patient was considered to have a dRVVT-LA profile.

Detection of anti-cardiolipin antibodies (aCL)

aCL were measured by a enzyme-linked immunosorbent assay (ELISA)[20] that in our laboratory has intra- and inter-assay coefficients of variation ranging from 6.1 to 9.7%.

Detection of anti-prothrombin antibodies (aPT)

aPT were measured using an ELISA developed in our laboratory.[21] ELISA, plates were previously irradiated and human prothrombin (Stago, Asnières, France) was used as antigen. Intra- and inter-assay coefficients of variation of this assay ranged from 4.6 to 13.3%.[21]

Detection of anti-β2-glycoprotein I antibodies (aβ2GPI)

aβ2GPI were measured using an in house ELISA previously reported.[22] In this assay irradiated plates were coated with purified human beta-2 glicoprotein I (Stago). Intra- and inter-assay coefficients of variation of this assay ranged from 7.0 to 12.1%.[22]

Activated protein C resistance

APCR was determined in plasma measuring the prolongation of activated partial thromboplastin time in response to the addition of activated protein C (Chromogenix, Mölndal, Sweden). Classical APCR test (undiluted test) and APCR test after sample dilution (1:5, vol/vol) with factor V deficient plasma containing normal protein S amount (Chromogenix). Results were expressed as ratios between activated partial thromboplastin time with and without activated protein C. Patients with a ratio lower than 2.0 were considered to have APCR.

Factor V Leiden determination

The presence of factor V Leiden mutation (Arg506Gln) was tested using polymerase chain reaction (PCR) amplification and allele specific oligonucleotide (ASO) hybridization (mD, Factor V Leiden, Bio-Rad Laboratories Diagnostics Group, Hercules, CA, USA).[23] Briefly, the region of the factor V DNA carrying the Leiden mutation (1691 G→A) is amplified using two specific 5′-biotinilated oligonucleotide primers. Detection was performed in a 96-well microplaque containing immobilized ASO probes specific for the wild or the mutant allele of the factor V gene. The hybridized ASO-biotin-labeled PCR products were mixed with streptavidin-horseradish peroxidase conjugate. After addition of the substrate, optical density was measured at 450 nm.

Protein C and protein S activity

Protein C activity was quantified by a colorimetric assay (Chromogenix). Free and total protein S were quantified by ELISA using specific monoclonal antibodies (Stago).

Statistical analysis

Results are shown as absolute number and percentages, as median and ranges or mean and s.d. Chi-squared tests were used for comparing qualitative

variables and Fisher's exact test was used when the number of patients in a subgoup was too small. Odds ratios (OR) were calculated and its 95% confidence intervals (95% CI) were reported. Patients with congenital APCR, due to factor V Leiden, were excluded in the analysis of acquired APCR and their relationship with aPL, thrombosis and miscarriage.

Results

Clinical characteristics

Major clinical manifestations of antiphospholipid syndrome in the whole series of SLE patients were the following: 20 (19%) patients had thromboses (10 arterial and 12 venous thromboses), and 26 females (40%) had pregnancy losses.

In addition, 25 (24%) patients developed thrombocytopenia (platelet count lower than 100×10^9/ml), 18 (18%) auto-immune hemolytic anemia, 15 (15%) nonrheumatic valve abnormality, and 1 (1%) chorea.

Prevalence of APCR

APCR in the undiluted test was present in 27 (26%) patients and APCR in the diluted assay was found in 21 (20%) of them. Factor V Leiden was detected in four (4%) patients and all of them were hererozygotes. Consequently, in SLE acquired APCR was found in 23 patients (22%) in the undiluted test and in 17 patients (17%) in the diluted test. Mean APCR value of positive patients was 1.74 (s.d. = 0.12; range = 1.59–1.98) in the direct test and 1.76 (s.d. = 0.14; range = 1.60–1.99) in the diluted test.

In controls, four individuals (4%) have APCR in both undiluted and diluted test. All these individuals had heterozygous factor V Leiden mutation.

Relationship between acquired APCR and aPL

Classical aPL (LA and/or aCL) were detected in 41 patients (40%). The prevalence of all the aPL, including those directed against cofactors, are shown in Table 1. In patients with classical aPL (LA and/or aCL) acquired APCR was more frequent than in those without aPL, in both the undiluted (39 *vs* 13%; $P = 0.007$; OR = 4.1, 95% CI = 1.5–10.8) and the diluted assay (33 *vs* 7%; $P = 0.001$; OR = 7.0, 95% CI = 2.1–23.5). The relationship between acquired APCR and aPL is described in Table 2. The aCL were significantly associated with acquired APCR in both the diluted and the undiluted tests, and IgG isotype of aPT was only associated with acquired APCR in the undiluted assay. No relationship was found between KCT- and dRVVT-LA profiles and APCR.

Table 1 Prevalence of antiphospholipid antibodies in patients with systemic lupus erythematosus ($n = 103$)

	n	%
Lupus anticoagulant	29	28
dRVVT[a] profile	12	12
KCT[b] profile	17	17
Anticardiolipin antibodies	29	28
IgG	23	22
IgM	12	12
Antiprothrombin antibodies	40	39
IgG	28	27
IgM	19	18
Anti-β2-glycoprotein I antibodies	25	24
IgG	15	15
IgM	17	17

[a]dRVVT, dilute Russell's viper venom time.
[b]KCT, Kaolin clotting time.

Relationship between acquired APCR and thrombosis

In patients with aPL (LA and/or aCL) thromboses were more frequent than in patients without aPL

Table 2 Relationship between acquired activated protein C resistance and antiphospholipid antibodies in patients with systemic lupus erythematosus ($n = 99$, excluding four patients with factor V Leiden)

Parameter (n)	*Undiluted APCR,[a] n (%)*	*Diluted APCR, n (%)*
LA[b] + (28)	10 (36)	8 (29)
LA − (71)	13 (18)	9 (13)
dRVVT[c]-LA + (11)	4 (36)	3 (27)
dRVVT[c]-LA − (88)	19 (22)	14 (16)
KCT-LA + (17)	6 (35)	5 (29)
KCT-LA − (82)	17 (21)	12 (15)
aCL[d] + (28)	12 (43)*	10 (36)*
aCL − (71)	11 (16)	7 (10)
aCL IgG + (23)	10 (44)**	9 (39)*
aCL IgG − (76)	13 (17)	8 (11)
aCL IgM + (11)	5 (46)	3 (27)
aCL IgM − (88)	18 (21)	14 (16)
aPT[e] + (40)	13 (33)	9 (23)
aPT − (59)	10 (17)	8 (14)
aPT IgG + (28)	11 (39)*	8 (29)
aPT IgG − (71)	12 (17)	9 (13)
aPT IgM + (19)	5 (26)	4 (21)
aPT IgM − (80)	18 (23)	13 (16)
aβ2GPI[f] + (25)	7 (28)	5 (20)
aβ2GPI − (74)	16 (22)	12 (16)
aβ2GPI IgG + (15)	4 (27)	4 (27)
aβ2GPI IgG − (84)	19 (23)	13 (16)
aβ2GPI IgM + (17)	7 (41)	5 (29)
aβ2GPI IgM − (82)	16 (20)	12 (15)

[a]APCR, activated protein C resistance.
[b]LA, lupus anticoagulant.
[c]dRVVT, dilute Russell's viper venom time.
[d]KCT, kaolin clotting time.
[e]aCL, anticardiolipin antibodies.
[f]aPT, antiprothrombin antibodies.
[g]aβ2GPI, anti-β2-glycoprotein I antibodies.
*$P < 0.01$.
**$P < 0.05$.

(34 *vs* 10%; $P=0.004$; OR = 4.8, 95% CI = 1.6–13.9). A significant association was found between acquired diluted APCR and arterial thrombosis (24% in patients with acquired APCR in comparison to 6% in patients without; $P=0.04$; OR = 4.7, 95% CI = 1.1–20.0). Table 3 shows the relationship between acquired APCR and thrombosis. One patient with factor V Leiden had two deep venous thrombosis in legs and one stroke.

Relationship between acquired APCR and miscarriage

Sixty-five women were previously pregnant among the 97 women with SLE. Twenty-six (40%) had history of pregnancy losses. Miscarriage was significantly associated with aPL (56% in women with aPL in comparison to 25% in women without; $P=0.01$; OR = 3.7, 95% CI = 1.2–10.9). In two women with previous pregnancy losses factor heterozygote V Leiden was found. Excluded these two patients, acquired APCR was found in 12 (18%) and in 10 (15%) women in the undiluted and in the diluted assay, respectively. Acquired APCR was found to be significantly associated with pregnancy losses, in both the undiluted and the diluted assay. In the undiluted assay 67% (8/12) of women with acquired APCR had pregnancy losses in comparison to 31% (16/51) in those without ($P=0.04$; OR = 4.3, 95% CI = 1.1–16.6). Moreover, in the diluted assay, 70% (7/10) of women with acquired APCR also had pregnancy losses in comparison to 32% (17/53) in those without ($P=0.03$; OR = 4.9, 95% CI = 1.1–21.4). Table 4 summarizes these results.

Protein C and protein S

No congenital deficiencies of protein C or protein S were detected in the present series of patients with SLE.

Table 3 Relationship between acquired activated protein C resistance and thrombosis in patients with systemic lupus erythematosus ($n=99$, excluding four patients with factor V Leiden)

	Arterial thrombosis, n (%)	*Venous thrombosis*, n (%)	*Total*, n (%)
Undiluted APCR[a]			
Positive (23)	4 (17)	1 (4)	5 (22)
Negative (76)	5 (7)	10 (13)	14 (18)
Diluted APCR			
Positive (17)	4 (24)*	1 (6)	5 (29)
Negative (82)	5 (6)	10 (12)	14 (17)

[a]APCR, activated protein C resistance.
*$P=0.04$.

Table 4 Relationship between acquired APCR and miscarriage in patients with systemic lupus erythematosus ($n=63$, excluding two patients with factor V Leiden)

	Miscarriage, n (%)	P	*OR*[a]
Undiluted APCR[b]			
Positive (12)	8 (67)	0.04	4.3
Negative (51)	16 (31)		
Diluted APCR			
Positive (10)	7 (70)	0.03	4.9
Negative (53)	17 (32)		

[a]Odds ratio.
[b]APCR, activated protein C resistance.

Discussion

In the present study we found a high prevalence of APCR in patients with SLE. In these patients, APCR was mainly acquired and not due to the presence of factor V Leiden. Acquired APCR was significantly associated with aPL. We also found association between acquired APCR and arterial thrombosis or miscarriage. These results suggest that acquired APCR may represent a mechanism of thrombosis in patients with SLE.

The prevalence of factor V Leiden in SLE patients (4%) was similar to the prevalence seen in the whole population[24,25] (4% in the present control series). Additionally, factor V Leiden has not been found the unique cause of APCR in patients with primary antiphospholipid syndrome.[26,27] The prevalence of acquired APCR has been reported to be higher in both patients with SLE[28] or primary antiphospholipid syndrome.[26] Recently, Male *et al.*[29] found a high prevalence of acquired APCR (31%) in pediatric SLE patients, similar to the prevalence observed in our study performed in adult population (22% in the undiluted assay and 17% after diluting the test plasma in factor V-deficient plasma). Therefore, the association between aPL and acquired APCR seems to be clear.

In the present series, when we analyzed the relationship between acquired APCR and aPL, including the antibodies directed against cofactors (aPT and aβ2GPI), a significant association was found with aCL but not with LA in the diluted APCR test, in agreement with a previous report.[30] However, other authors have not observed this relationship.[29,31,32] Previous reports suggested that the dRVVT-LA profile (theoretically more sensitive to the presence of aβ2GPI) but not the KCT-LA profile (theoretically more sensitive to the presence of aPT) is associated with thrombosis.[33] In the present series, although acquired APCR was related to arterial thrombosis, no relationship was found between KCT- or dRVVT-

LA profiles and acquired APCR. These findings seem to support that acquired APCR is not mainly due to an *in vitro* interference of LA, but it is a true acquired APCR that may contribute to a prothrombotic state in SLE patients with aPL.

The mechanism causing APCR in aPL positive SLE patients is not clear, although it has been suggested to be related with a functional interference of aPL with the protein C pathway rather than a depletion of their components.[29] The inactivation of factor Va by activated protein C in the presence of protein S needs phospholipids and calcium.[34] It has been reported that aPL may prevent the inactivation of factor Va by activated protein C,[8] and it has been speculated that the impairment of catalytic activity of activated protein C would be the result of a reduction in the number of available sites on phospholipids due to the binding of aPL.[35]

In the present series of SLE patients we found a significant association between acquired APCR and thrombosis. The present is the first study in adults showing this relationship in patients with SLE, in agreement with the results obtained in a pediatric population by Male *et al.*[29] We found this association only with arterial thromboses, but not with venous thromboses. A comparison between our data and those from the study of Male *et al.* is not possible because in the later thrombosis are described but a separate analysis of the relationship between acquired APCR and arterial or venous thromboses was not performed. Congenital APCR is usually associated with venous thrombosis but we found the association with arterial thrombosis. There are some studies reporting a relationship between APCR and arterial disease in both acquired[36,37] and congenital APCR.[38–40] However, in the majority of these studies APCR needs to be associated with other risk factors to increase arterial thrombosis. In the present series, SLE and/or aPL can be these additional risk factors. In addition, APCR due to factor V Leiden and acquired APCR seen in SLE may express two different forms of interfering in protein C anticoagulant system that could have a different clinical expression. However, our results must be interpreted cautiously because thromboses were identified retrospectively and in a relatively small number of patients. In addition, our findings may not be directly extrapolated to other diseases like the primary antiphospholipid syndrome, where the relationship between acquired APCR and thrombosis have been suggested,[41,42] and several methods have been proposed to differentiate the true APCR (acquired or congenital) to the spurious APCR phenotype due to a unspecific prolongation in the coagulation tests by LA not related to its effect in protein C activity.[41,43]

On the other hand, we also observed an association between pregnancy losses and acquired APCR. Thrombophilia has been identified as a possible factor in recurrent pregnancy losses.[44] In this way, recent studies have shown that both acquired and congenital APCR is related to recurrent miscarriage in women without underlying autoimmune disease.[45–48]

In conclusion, in SLE patients acquired APCR seems to be associated with increased prevalence of arterial thrombosis and pregnancy losses.

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RESUMEN

Introducción: Los anticuerpos antifosfolipídicos pueden generar una resistencia adquirida a la acción de la proteína C activada y esta resistencia adquirida podría ser un mecanismo causante de trombosis, aunque su papel en pacientes con lupus eritematoso sistémico no está claro.

Objetivo: Evaluar la prevalencia de la resistencia adquirida a la proteína C activada en pacientes con lupus eritematoso sistémico y analizar su posible relación con los anticuerpos antifosfolipídicos y con las principales manifestaciones clínicas asociadas a los mismos: trombosis y abortos o pérdidas fetales.

Material y métodos: Se estudiaron 103 pacientes con lupus eritematoso sistémico (97 mujeres y 6 hombres; edad media de 38 años). Se incluyó un grupo control formado por 103 voluntarios sanos. La resistencia a la proteína C activada se determinó en el plasma midiendo la prolongación del tiempo de tromboplastina parcial activada (TTPA) en respuesta a la adición de proteína C activada. El ensayo se realizó primero sin dilución de la muestra y posteriormente diluyéndola en plasma deficitario en factor V. Los resultados se expresaron como el cociente del TTPA determinado tras la adición de proteína C activada dividido por el TTPA determinado sin su adición. Además se estudió la presencia del factor V Leiden mediante PCR. El anticoagulante lúpico se evaluó mediante ensayos coagulométricos y los anticuerpos anticardiolipina, antiprotrombina y anti-β2 glicoproteína I mediante técnicas de ELISA.

Resultados: La prevalencia del factor V Leiden fue del 4%, igual a la encontrada en el grupo control. La prevalencia de la resistencia adquirida a la proteína C activada (no debida al factor V Leiden) fue del 22% en el ensayo sin dilución y del 17% tras la dilución en plasma deficitario en factor V. Esta resistencia adquirida se encontró más frecuentemente en pacientes con lupus y anticuerpos antifosfolipídicos que en aquellos sin anticuerpos antifosfolipídicos (39% vs 13% en el ensayo sin dilución, p=0,007; 33% vs 7% en el ensayo diluido, p=0,001). Se observó una asociación entre resistencia adquirida a la proteína C activada y los anticuerpos anticardiolipina en ambos ensayos. También la resistencia adquirida a la proteína C activada se asoció con el isotipo IgG de los anticuerpos antiprotrombina, aunque sólo en el ensayo sin dilución de las muestras. Las trombosis arteriales fueron más frecuentes en pacientes con resistencia adquirida a la proteína C activada determinada tras la dilución de la muestra en plasma deficitario en factor V (24% vs 6%; p=0,04). No se observó relación con las trombosis venosas. Los abortos o pérdidas fetales también fueron más frecuentes en mujeres que mostraron dicha resistencia adquirida, tanto en el ensayo sin diluir (67% vs 31%; p=0,04) como tras la dilución en plasma deficitario en factor V (70% vs 32%; p=0,03).

Conclusiones:

1. En pacientes con lupus eritematoso sistémico la resistencia a la proteína C activada debida a la presencia del factor V Leiden es similar a la de la población general.

2. La resistencia adquirida a la proteína C activada alcanza una prevalencia alrededor del 20% y se asocia de forma significativa con la presencia de los anticuerpos antifosfolipídicos, específicamente con el isotipo IgG de los anticuerpos anticardiolipina.
3. La resistencia adquirida a la proteína C activada se asocia a una mayor prevalencia de trombosis arteriales y de fracasos de los embarazos en pacientes con lupus eritematoso sistémico.