The role of lupus anticoagulant and triple marker positivity as risk factors for rethrombosis in patients with primary antiphospholipid syndrome

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Abstract

Objective

To ascertain rethrombotic risk factors in patients with primary antiphospholipid syndrome (PAPS).

Methods

We retrospectively evaluated 95 patients according to their rethrombotic status. We registered anticoagulation (OA) status, comorbidities, traditional thrombotic factors, prevalence of aCL (IgG-IgM), anti-β_2GP-I (IgG-IgM), LA and triple marker positivity (LA, aCL and anti-β_2GP-I).

Results

Forty-two patients had rethrombosis and 53 were rethrombosis-free. The median follow-up was 4.5 (0.3–26) years. There were no differences in comorbidities and traditional thrombotic factors. Patients with rethrombosis had more frequently LA (62% vs. 40%, p=0.04), were younger (41 vs. 47 years, p=0.01) and received less frequently OA (23% vs. 54%, p=0.002). A logistic regression analysis showed that the OA status (OR 0.17, 95% CI 0.05–0.57, p=0.004) and age (OR 0.94, 95% CI 0.90–0.98, p=0.01) remained significant. Patients who discontinued OA and developed rethrombosis (Group 1, n=32) vs. patients who discontinued OA, but remained rethrombosis-free (Group 2, n=24) were also analysed. We found a higher prevalence of LA and triple marker positivity in Group 1 (67% vs. 31%; OR= 4.5, 95% CI 1.3–14.9, p= 0.01 and 57% vs. 27%; OR 3.6, 95% CI 1.7–12; p=0.03), respectively. Both variables remained associated with rethrombosis when compared with the overall rethrombosis group vs. Group 2 (LA 62% vs. 31%, OR= 3.6 95% CI 1.1–11.2, p=0.03; triple marker 54% vs. 27%; OR 32 95% CI 1.01–10.2, p=0.05).

Conclusion

LA positivity and triple aPL positivity confer a more severe risk of rethrombosis in PAPS patients, irrespective of their anticoagulation status and known conventional risk factors.

Keywords

antiphospholipid syndrome, rethrombosis, oral anticoagulation, lupus anticogulant, triple marker

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Introduction

Retrospective studies have shown recurrence of thrombotic events in around 70% of patients with antiphospholipid syndrome (APS). For instance, Khamashta *et al*. reported that the recurrence rate per patient-year for a six-month period is of 1.3 after oral anticoagulant (OA) discontinuation (1). This incidence of recurrences highlights that APS patients should receive long-term anticoagulant treatment (2, 3). It is also known, however, that the recurrence of thrombosis remains despite the use of OA regardless of its intensity (4). For instance, the rate of thrombotic recurrences in patients with INR between 2.1 and 2.6 has been reported in 9.1 cases per 100 patient-years in patients on OA (5), the majority of which occurs in the same vascular bed (5, 6).

According to the Sydney criteria for the classification of APS, a single persistently positive lupus anticoagulant (LA), anticardiolipin (aCL) and/or antibodies to β_2-glycoprotein-I (anti-β_2GP-I) fulfill the serologic criteria (7). More recently, however, the concept of a triple positivity of these antibodies has been proposed to identify patients with a higher risk for developing thromboembolic events despite the use of oral anticoagulants (6). For instance, triple positivity is an independent risk factor for pregnancy failure in women with APS compared to patients with a single positive test (OR=4.1; 95% CI 1.0–16.7; p=0.05) (8). This antibody profile also confers a higher risk for future thromboembolic episodes in asymptomatic carriers (9). More recently, Govoni *et al*. found by multiple stepwise logistic regression analysis, that APS and the simultaneous presence of aCL, anti-β_2GP-I and LA were independently related to central nervous involvement, particularly cerebrovascular events, in a large cohort of Italian patients with systemic lupus erythematosus (SLE) (10).

Other non-serological risk factors for thrombosis have also been studied in APS. Thus, hypertension appears to be a risk factor for the first arterial event in both primary (11-13) and secondary APS (14). For instance, Ruffatti *et al*. reported that antiphospholipid (aPL) carriers with hypertension have a hazard ratio of 3.8 (CI 95% 1.3–11) for developing a first thrombotic event (14). Other authors have found inconsistent data regarding smoking, previous arterial episodes (11), hypertriglyceridemia and hereditary thrombophilia in the appearance of venous thrombotic events (13) as well as between hypocomplementaemia and obstetric complications in women with APS (15). Finally, variables such as diabetes mellitus, hypercholesterolemia, oestrogens, surgical procedures, pregnancy, malignancy, infections or thrombocytopenia have not been associated with a first thrombotic event in primary (11) or secondary APS (13).

The objective of our study was, therefore, to better understand the role of serological (LA, aCL and anti-β_2GP-I antibodies, alone or in combination) and non-serological risk factors for recurrence of thrombosis in a retrospective cohort of patients with primary APS, regardless of their anticoagulation status.

Methods

We reviewed the medical records of 95 consecutive, unselected patients with primary APS who from January 1986 to December 2009 attended the Department of Immunology of Rheumatology of the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, a tertiary referral care centre. To be included patients should have had at least one thrombotic episode (7) and two or more positive determinations, 12 weeks apart, of IgG or IgM aCL, or IgG or IgM anti-β_2GP-I (16) or positive LA (17). Patients were excluded if they had a known hereditary thrombophilia or fulfilled any serological and/or clinical criteria for SLE (18).

Follow-up time was considered from the first thrombotic event until the next episode of thrombosis. If patients did not develop a new episode of thrombosis, follow-up was calculated up to the last medical appointment (LMA). All patients received OA after the first thrombotic event. During follow up, however, some patients *motu proprio* discontinued OA, others due to an adverse event or some others as per their treating physician's recommen-

dations. Patients' clinical records were carefully reviewed according to a pre-established protocol. We collected demographic features, type of thrombosis and rethrombosis (confirmed by image diagnostic methods or biopsy), time to rethrombosis, LMA, body mass index (BMI), anticoagulation status and INR at rethrombosis or LMA if patient was still on OA. We also evaluated conventional thrombosis risk factors such as pregnancy, bedridden, trauma, estrogen replacement, nephrotic syndrome, malignancy and smoking. Comorbidities such as diabetes mellitus, hypertension and dyslipidemia, and levels of triglycerides, HDL, LDL at the time of rethrombosis or at LMA, if rethrombosis-free, were also evaluated. We also registered infections (documented by culture or during a hospitalisation or clinic visit), use of prednisone, immunosuppresors and aspirin. We analysed the prevalence and titers of aCL (IgG-IgM), anti-β_2GP-I (IgG-IgM), LA and the combination of the three (triple serologic marker) during follow up. We also studied the persistency of aCL and anti-β_2GP-I antibodies (all isotypes), defined as the positivity of aPL in at least 75% of ≥3 available determinations per patient during follow-up.

Finally, we classified patients in four groups according to their anticoagulation and rethrombosis status (Fig. 1). Group 1: patients who discontinued OA treatment and developed a new thrombotic event during follow up. Group 2: patients who also discontinued OA but remained rethrombotic-free at follow up. Group 3: patients on OA that remained rethrombosis-free at follow-up. Group 4: patients who despite being on OA developed a new thrombotic event during follow up. We were particularly interested in patients in Group 2 so we compared relevant variables of this group vs. Group 1 and *vs*. Groups 1 + 4 (rethrombosis groups, regardless OA status). In addition we also compared Group 3 *vs*. 4.

Antiphospholipid assays

aCL (IgG and IgM) were determined by ELISA according to published methods (16). IgG and IgM antibodies to phospholipid-free human β_2GP-I were determined by ELISA according to Cabiedes *et al*. (16). Lupus anticoagulant was determined by coagulation tests using platelet-poor plasma according to the guidelines of the International Society on Thrombosis and Haemostasis (Scientific Subcommittee on Lupus Anticoagulants/Phospholipid-Dependent Antibodies) (17).

Cut-off points for aCL or anti-β_2GP-I ELISAs at time of study were considered positive according to reference values in use during the study period at the Immunology and Rheumatology Laboratory of our Institution following published criteria from our group (16, 19, 20).

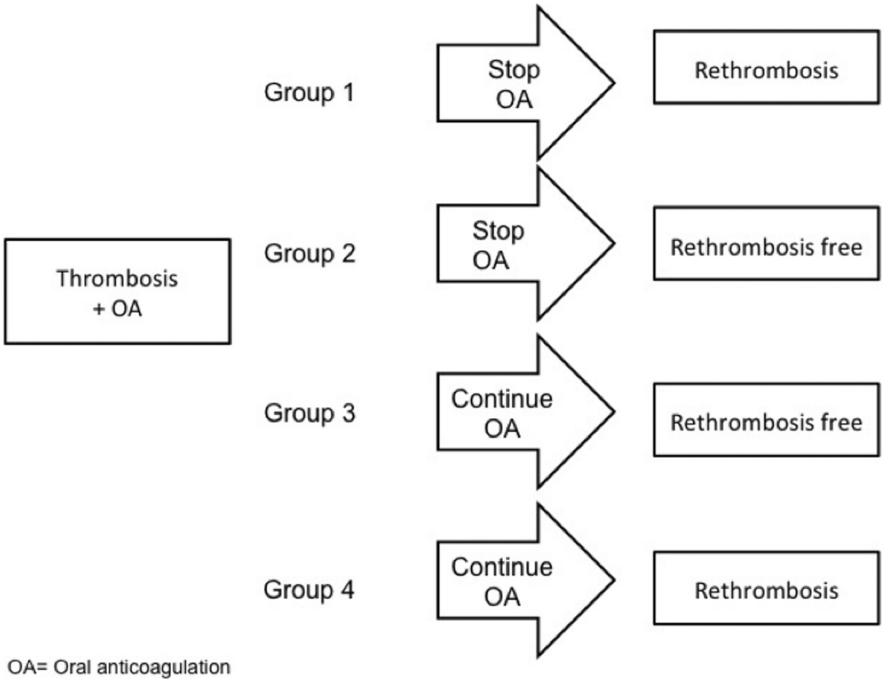

Fig. 1. Study groups according the rethrombosis and anticoagulation status.

Statistical analysis

Categorical variables were compared using either χ^2 or Fisher's exact test when appropriate, continuous variables were compared using Student's *t*-test and Mann-Whitney U-test when non-normally distributed. Significant variables in the univariate analysis ($p<0.10$) were tested by way of a regression logistic model. Odds ratios (OR) are reported with 95% confidence intervals. We also calculated the probability of being free of rethrombosis using Kaplan-Meier survival curves and compared the survival rethrombosis curves among patients with and without OA using the Breslow test.

Finally, we performed pair-wise comparison of relevant variables between Group 1 (without OA and rethrombosis) *vs*. Group 2 (without OA and rethrombosis free), between Group 1+4 (rethrombosis regardless or anticoagulation status) *vs*. Group 2 (without OA and rethrombosis free), and as well as between Group 3 *vs*. 4 (rethrombosis/or not rethrombosis in the presence of OA). A two-tailed $p<0.05$ was considered significant. All analyses were performed using SPSS for Windows 17.0® (SPSS Inc).

Results

We studied 95 patients (70 women, 74%) with a mean age at time of study of 41.7±14 years and with a median follow-up of 4.5 years (0.3-26). Forty-two patients (44%) had a rethrombotic event whereas 53 (55%) patients did not. Table I shows the distribution of demographic data, treatments, sites of thrombosis and rethrombosis amongst the study groups. We did not find any statistical difference in these variables with the exception of a longer follow-up (5.7 years *vs*. 3 years, $p=0.01$) as well as an older age (47 years *vs*. 41 years, $p=0.01$) in the rethrombosis-free group.

Table I. Demographic and clinical variables.

Variable	Rethrombosis n=42	Rethrombosis free n=53	*p*-value
Women, %	27 (64.3)	43 (81)	0.06
Age in years	41 ± 15.2	47 ± 14	0.01
Follow up in years	3 (0.68-20.2)	5.7 (1.1-21)	0.01
Thrombosis			0.16
Arterial	11	21	
Venous	31	32	
Rethrombosis			
Arterial	21	Not applicable	Not applicable
Venous	21		
Prednisone,%	4 (9.5)	3 (5.7)	0.69
Immunosuppresors,%	2 (4.8)	2 (3.8)	1
Antimalarial, %	1 (2.4)	0	0.42
Aspirin,%	21 (50)	24 (45.3)	0.64
Oral anticoagulation	10 (23.8)	29 (54.7)	0.002

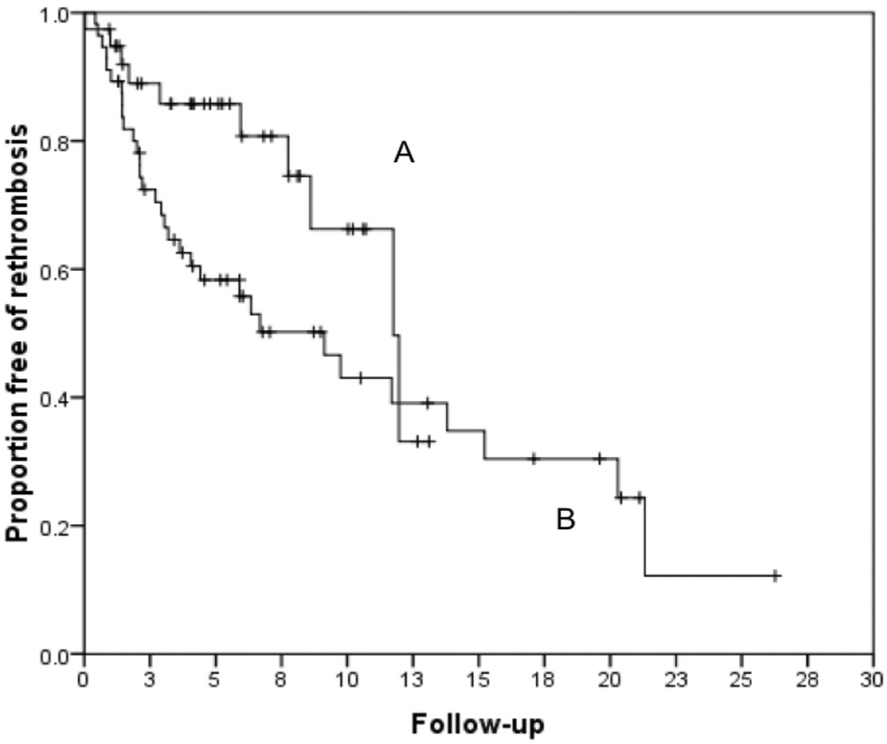

Fig. 2. Kaplan-Meir curves of the probability of absence of rethrombosis in two study groups: with oral anticoagulation (A) and without oral anticoagulation (B).

The use of aspirin was similar among groups (50% in the rethrombosis group and 45% in the rethrombosis-free group).

As expected, patients in the rethrombosis-free group were more frequently on OA (54.7% *vs*. 23.8%, p=0.002). Figure 2 shows the Kaplan-Meier curves of the cumulative survival free of rethrombosis at different points of time in patients who continued under OA compared with patients that did not (Breslow test: 4.7 p=0.02). The median time of rethrombosis in anticoagulated patients was 11.7 years (95% CI 8.4–15) *vs*. 9.1 (95% CI 4.6–13.6) in patients without anticoagulant treatment.

We did not find any differences in the frequency of comorbidities or any other clinical risk factors among groups (Table II). The distribution of prevalence of aPL amongst the different groups is shown in Table III. Overall, the most prevalent antibody was IgM aCL in 83 patients (87.4%), followed by IgG anti-β_2GP-I in 71 patients (78%), IgM anti-β_2GP-I in 59/81 (72%) patients, IgG aCL in 55 patients (57.9%), LA in 40/80 patients (50%) and triple marker in 44% (34/77). Similarly the persistency of the aPL antibodies were: IgM aCL in 72% (67/92 patients), IgG anti-β_2GP-I in 46% (36/78 patients), IgM anti-β_2GP-I in 45% (27/59 patients), and IgG aCL in 36% (34/93 patients). Rethrombosis-free patients had less frequently LA (40% *vs*. 62.9%, p=0.04) and a lower prevalence of a triple-positive serology (36.4% *vs*. 54.5%), this latter, however, was not statistically significant. The persistency of IgM aCL was significantly higher in the rethrombosis-free group (82% *vs*. 60%, p=0.01). No difference in any antibody titer was found (data not shown).

The logistic regression analysis showed that the only variables that remained significant were the anticoagulation status (OR 0.17, 95% CI 0.05–0.57, p=0.004) and age (OR 0.94, 95% CI 0.90–0.98, p=0.01).

Finally, we analysed patients according to their anticoagulation and rethrombosis status as defined above. Patients were distributed amongst the following groups: 32 patients in Group 1, 24 in Group 2, 29 in Group 3 and 10 in Group 4. As previously found in the first overall analysis, patients were similar in the presence of traditional thrombosis risk factors (data not shown) and comorbidities. Nevertheless, the follow-up time was shorter in Group 1 (G1=2.8 years, G2=5.9 years, G3=5.1 years and G4=4.4 years), p<0.05). Among patients who continue on OA, the INR level at rethrombosis (Group 4) or at the last medical appointment (Group 3) was similar (2.3±1 *vs*. 2.7±1, respectively). The subgroups serology is shown in Table IV.

We also compared patients who developed a new episode of thrombosis (Group 1) with those patients that remained thrombosis-free during follow-up (Group 2), both groups without oral anticoagulants. We found that LA was significantly more prevalent in Group 1 (19/28 (67%) vs. 7/22(31%), OR=4.5; 95% CI=1.3–14.9; p=0.01) as well as the triple marker positivity (15/26 (57%) *vs*. 6/22 (27%); OR=3.6, 1.7–12.2; p=0.03). These two variables remained associated with recurrence of thrombosis after comparing Groups

Table II. Comorbidities and traditional thrombotic risk factors.

Variable	Rethrombosis n=42	Rethrombosis free n=53	*p*-value
Diabetes mellitus, %	4 (9.5)	4 (7.5)	0.73
Dyslipidemia, %	4 (9.5)	11 (20.8)	0.13
Triglycerides, mg/dl	141 (48-217)	146 (45-572)	0.89
HDL, mg/dl	38 (18-67)	39.5 (20-75)	0.54
LDL, mg/dl	97 (48-150)	109 (53-186)	0.42
Hypertension, %	13 (31)	14 (26.4)	0.62
Estrogen use, %	0	0	1
Pregnancy, %	0	0	1
Nephrotic syndrome, %	0	0	1
Malignancy, %	2 (4.8)	0	0.19
Smoking, %	9 (21.4)	6 (11.3)	0.18
Bedridden, %	3 (7.1)	3 (5.7)	0.51
Trauma, %	0	1 (1.9)	1
Hepatitis B or C, %	0	1 (1.9)	1
Inpatient infection, %	5 (11.9)	3 (5.7)	0.45
Ambulatory infection, %	4 (9.5)	2 (3.8)	0.42
Body mass index	26.3 ± 5	27.8 ± 3.7	0.32

Table III. aPL distribution.

	Rethrombosis n=42	Rethrombosis free n=53	*p*-value
IgG aCL			
Prevalence, %	24 (57.1)	31 (58.5)	1
Persistency, %	16/41 (39)	18/52 (34)	0.72
IgM aCL			
Prevalence, %	36 (85.7)	47 (88.7)	0.6
Persistency, %	24/40 (60)	43/52 (82)	0.01
IgG anti-β_2GP-I			
Prevalence, %	30/40 (75)	41/51 (80.4)	0.5
Persistency, %	15/34 (44)	21/44 (47)	0.29
IgM anti-β_2GP-I			
Prevalence, %	22/36 (61)	35/45 (77)	0.1
Persistency, %	9/13 (69)	18/36 (50)	0.41
LA, %	22/35 (62.9)	18/45 (40)	0.04
Triple marker, %	18/33 (54.5)	16/44 (36.4)	0.10

Table IV. aPL antibodies by subgroups.

	Group 1 n= 32	Group 2 n=24	Group 3 n=29	Group 4 n=10
IgG aCL				
Prevalence, %	18 (56)	10 (41.7)	21 (72)	6 (60)
Persistency, %	13/31 (41)	5/23 (44)	13/29 (44)	3/10 (30)
IgM aCL				
Prevalence, %	29 (90)	20 (83)	27 (93)	7 (70)
Persistency, %	18/30 (60)	18/23 (78)	25/29 (86)	6/10 (60)
IgG anti-β_2GP-I				
Prevalence, %	22/30 (73)	19 (79)	22/27 (81)	8 (80)
Persistency, %	13/25 (52)	9/21 (42)	12/23 (52)	2/9 (22)
IgM anti-β_2GP-I				
Prevalence, %	16/28 (57)	16/22 (72)	19/23 (82)	6/8 (75)
Persistency, %	7/9 (36)	8/17 (47)	10/19 (52)	2/4 (50)
LA, %	19/28 (67)	7/22 (31)	11/23 (47)	3/7 (42)
Triple marker, %	15/26 (57)	6/22 (27)	10/22 (45)	3/7 (42)

1 + 4 (patients with rethrombosis with or without OA) *vs*. Group 2 (LA 22/35 (62%) *vs*. 7/22 (31%), OR 3.6, 95% CI 1.1–11.2, *p*=0.03; triple marker (18/24 (54.5%) *vs*. 6/22 (27%), OR=3.2 95% CI 1.04–10.2; *p*=0.05, respectively). Finally when we compared group 3 *vs*. 4 we did not find any significant difference with the exception of a tendency of a higher frequency of aCL IgM isotype (93% *vs*. 70%, *p*=0.09) in Group 3; however it is important to underline that Group 4 only comprised 10 patients.

Discussion

The pathophysiology of thrombosis in APS is complex and multi-factorial. The interaction between genetic factors, environmental components and the presence and type of aPL antibodies would presumably determine whether and when an individual will suffer a thrombotic event (21). In order to identify patients with serological risk factors, the combination of different aPL in the same patient has been proposed. For instance, Pengo *et al*. proposed that patients with triple positivity (LA, aCL and anti-β_2GP-I) have the highest risk for developing thromboembolic events (6, 22). Other authors previously found that LA and the simultaneous and constantly high presence of different aPL antibodies in lupus patients are major factors influencing the development of clinical thrombosis (23). In view of these reports, the main objective of our study was to ascertain whether aCL, anti-β_2GP-I and LA, alone or in combination, anticoagulant treatment and conventional venous and arterial factors confer a higher risk for rethrombosis in PAPS patients. We studied patients with at least one thrombotic episode with or without oral anticoagulants that did or did not develop a new episode of thrombosis during follow-up. We found that the presence of LA and lack of OA treatment were associated with a rethrombotic state. We also found that LA combined with aCL and anti-β_2GP-I (triple positivity) increased the odds of thrombotic recurrences. These findings are in agreement with the notion that LA confers a high risk for developing thrombosis in APS patients (24). These results also agree with the notion that APS patients with triple positivity for aPL are at risk of developing future thromboembolic events (6), higher risk of pregnancy failure in women with APS (8) and cerebrovascular events in patients with SLE (10). Our report extends these observations to now include higher risk of rethrombosis in patients with definite primary APS.

It has been long known that IgG anti-β_2GP-I is an independent risk factor for developing thrombosis in both primary and secondary APS (16, 25). In the current study, IgG anti-β_2GP-I had the same prevalence between groups. In this regard, group 2 represents a peculiar and challenging subset of patients. The majority of these individuals (79%), who altogether had a history of 24 thrombotic events, have positive IgG anti-β_2GP-I, 9/21 (42%) with persistently positive titers, only 7/22 (31%) and 6/22 (27%) are LA- and triple-positive, respectively, but have remained thrombotic-free after a mean 6 years of follow-up. In the Leiden Thrombophilia Study, authors measured LA, anti-β_2GP-I and antiprothrombin and studied the risk of a first episode of deep venous thrombosis (DVT) in 473 patients and 472 control subjects (26). Authors found that LA, anti-β_2GP-I and aPT conferred a risk of 3.6, 2.4 and 1.4 for DVT, respectively. Interestingly, the risk increased to 10.1 when LA was assessed co-present with anti-β_2GP-I, but the risk was totally abolished when LA was studied in combination with negative anti-β_2GP-I (26). In view of these data, we speculate that anti-β_2GP-I in patients from the rethrombosis-free groups (Groups 2 and 3) and negative LA, may belong to a different subpopulation of non-thrombotic anti-β_2GP-I antibodies, perhaps with fine non-I domain epitope-binding specificity (27). This proposal is obviously amenable to further experimental testing.

The two-hit theory postulates that the presence of a second-trigger is necessary to activate the prothrombotic properties of aPL antibodies (11). In this context some studies have explored the conventional risk factors for the first thrombotic event (11) (13, 14). For instance, Erkan *et al*. evaluated thrombotic risk factors in 77 APS patients with established thrombotic events and compared them with 56 asymptomatic aPL-patients. Authors found that hypertension and smoking were associated with arterial events (11). Other researchers have confirmed the roll of hypertension (14), even in the setting of SLE and secondary APS (13). Thus, another objective of our study was to ascertain if conventional risk factors could have an influence in the development of rethrombosis in PAPS patients. Our results showed that hypertension, diabetes mellitus, cancer, dyslipidemia, infection, bedridden, trauma and estrogen use do not have any bearing in the development of rethrombosis in PAPS patients. This is in agreement with Danowski *et al*. who found that, with the exception of hypertension and elevated tryglicerides, conventional risk factors were not associated with the first venous thrombotic event (13).

Our results also allow us to highlight some relevant points regarding the secondary thromboprophylaxis in PAPS patients. Firstly, almost half of our patients were on aspirin regardless of their rethrombosis status. This, however, was not effective as a secondary anti-thrombotic prophylactic treatment. This result is in accordance with published reports (9, 28), including that of a large cohort of European APS patients in which a low-dose aspirin did not prevent the development of thrombosis (29). Secondly, there are no convincing data regarding the optimal duration of therapy or when anticoagulation may be discontinued in APS patients (3). Here we found that half of our patients on OA had a rethrombotic event 9 years after the first thrombosis, whereas half of the patients without OA had a rethrombosis 11 years after the first thrombotic event, this difference was statistically significant. This is not surprising since it is known that the risk of thrombotic recurrences is high even in patients on high-intensity oral anticoagulation (INR=3.5) for longer periods (5, 30). We found that the INR was similar among patients that despite being on OA suffered a new episode of thrombosis during follow-up compared with patients that did not suffer thrombotic recurrences. This is in agreement with other reports (31).

We acknowledge that our study has some limitations. Firstly, its retrospective design may have lead to selection and information biases. Secondly, we did not analyse other risk factors such as genetics of aPL antibodies and the presence of thrombophilia. Thirdly, the number of patients in some groups was low to have power to analyse by arterial or venous territories. Finally, our results derive from patients attending a tertiary referral center.

In conclusion, we did not find any non-serologic risk factor associated with rethrombosis in patients with PAPS. The group of PAPS patients free of rethrombosis even without OA treatment had less LA and triple marker positivity. This finding reinforces the concept that patients with LA and the triple positive population have a more severe course of the disease.

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