FACTORS ASSOCIATED WITH THE DEEP VEIN THROMBOSIS IN PATIENTS WITH ANTIPHOSPHOLIPID SYNDROME

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Abstract

Introduction. Patients with antiphospholipid syndrome (APS) may have a large spectrum of thrombotic clinical events (arterial or venous), but the factors that determine the occurrence of specific clinical manifestation has not been clearly established.

Objective. The aim of the study was to determine the factors associated with a history of deep vein thrombosis (DVT) in patients with APS. We were especially interested to find an association between the criteria and non-criteria antiphospholipid antibodies (APLAs) and the DVT.

Methods. We realized a cross-sectional study, with consecutive enrollment of all patients presented in our department with the diagnosis of APS in the period 2008-2011. From the total of 106 patients for which the demographic, clinical and biological parameters were collected, the assessment of the criteria and non-criteria APLAs was performed in 73 patients.

Results. The mean age at inclusion was 44.7 years, the female-to-male ratio 6.6, and the mean APS disease duration 6.7 years. The majority of the patients included presented with secondary APS (70 patients). Lupus anticoagulant was the most frequent immunological marker used to sustain the diagnosis of APS, found positive in 102 patients (96.2%). The recurrent DVT was more frequently observed in patients with primary APS than in those with secondary APS (p = 0.02). No significant association with DVT was found for the clinical parameters traditionally associated with the risk of thrombosis that we have taken into study: smoking, body mass index (BMI), waist circumference and waist-to-hip ratio. We found a positive association between the DVT history and the positivity for the IgM anti-β2 glycoprotein I antibodies [OR (95%CI) = 6.95 (1.36-35.58), p = 0.01]. The titer of IgG antiprothrombin antibodies and IgG antiphosphatidylethanolamine antibodies was higher in patients with previous DVT [3.0 (0.0-151.0) versus 2.0 (0.0-2.0), p = 0.01, respectively 5.0 (1.0-33.0) versus 3.0 (1.0-7.0), p = 0.01]. In the subgroup of patients with secondary APS, the previous DVT was associated only with the positivity for IgM anti-β2 glycoprotein I antibodies [OR (95%) = 13.63 (1.46-127.15), p = 0.02]. In the same subgroup, the levels of IgG anticardiolipin and the IgG antiphosphatidylethanolamine antibodies were higher in patients with previous DVT [2.0 (0.0-64.0) versus 1.0 (0.0-20.0), p = 0.02, respectively 3.0 (0.0-151.0) versus 2.0 (0.0-7.0), p = 0.03]. In patients with primary APS, the titer of IgG antiprothrombin antibodies was significantly higher when DVT history were present [5.0 (1.0-3.0) versus 3.0 (1.0-7.0), p = 0.01. Only the titer of IgG antiprothrombin antibodies was associated with the history of recurrent thrombosis [5.0 (1.0-3.0) versus 3.0 (1.0-7.0), p = 0.01].

Conclusions. In patients with APS, the DVT history was related with the prevalence of the anti-β2 glycoprotein I antibodies, but also with the levels of some non-criteria antibodies, IgG antiprothrombin antibodies and IgG antiphosphatidylethanolamine antibodies. In patients with primary APS, previous DVT is associated only with the titer of IgG antiprothrombin antibodies, whereas in patients with secondary APS, the DVT history were related with the presence of anti-β2 glycoprotein I antibodies and with the titers of both criteria antibodies (IgG anticardiolipin antibodies) and non-criteria antibodies (IgG antiphosphatidylethanolamine antibodies). The risk of recurrent thrombosis was higher in patients with primary APS when compared with those with secondary APS. Only the titers of IgG antiprothrombin antibodies were correlated with recurrent thrombosis.

Keywords: antiphospholipid syndrome, antiphospholipid antibodies, deep venous thrombosis

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Abbreviations
Abs – antibodies; **APLAs** – antiphospholipid antibodies; **aCL** – anticardiolipin; **aβ2GPI** – anti-β2 glycoprotein I; **aPE** – antiphosphatidylethanolamine; **aPS** – antiphosphatidylserine; **aPS/PT Abs** – anti-prothrombin in complex with phosphatidylserine; **aPT** – antiprothrombin; **APS** – antiphospholipid syndrome; **DVT** – deep venous thrombosis; **Ig** – immunoglobulin; **LAC** – lupus anticoagulant; **SLE** – systemic lupus erythematosus

INTRODUCTION

The diagnostic of antiphospholipid syndrome (APS) is mainly based on classification criteria released in 2006 (1): for a positive diagnostic at least one clinical and one laboratory criterion is mandatory. Thus, the APS classification criteria include a large number of clinical events (arterial or venous events and pregnancy morbidity). However, the clinical and laboratory parameters that determine the occurrence of arterial and venous events in patients with APS have not been clearly established.

Apart from the criteria antiphospholipid antibodies (APLAs) recognized by Sydney 2006 consensus statement, other APLAs were studied in patients APS. However, the significance for pathogenesis and utility in daily clinical practice of these non-diagnostic or non-criteria APLAs has not been defined yet.

The aim of this study was to establish the factors associated with a history of deep venous thrombosis (DVT) in patients with primary and secondary APS. Especially, we were interested to see if the positive determinations or the titers of criteria and non-criteria APLAs are associated with previous DVT.

METHODS

Study population

We performed a cross-sectional study, with consecutive enrollment of the patients presenting with the diagnostic of APS in Internal Medicine Department of Colentina Clinical Hospital in the period of 2008-2011.

We excluded from analysis the patients with chronic or acute infectious diseases (at the moment of enrollment or three months previously), granulomatous diseases, age under 18 years and pregnancy or post-partum period (for a period of 6 months).

All the available data prior to enrollment were analyzed in order to confirm patients' eligibility. A total of 106 patients diagnosed with primary or secondary APS were so recruited for this study.

This study was approved by the Clinical Research Ethics Committee of the "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania. All the patients agreed and signed an informed consent.

Variables

For all the patients included in study, demographic characteristics and history of APS (age and clinical manifestations at diagnostic and during the course of the disease, specific treatment and complications) were collected. Clinical parameters traditionally associated with the risk of thrombosis like smoking, body mass index (BMI), waist circumference and waist-to-hip ratio were also noted.

Blood samples were collected for all patients and then, after refrigeration at -70˚C, we performed the evaluation of specific antibodies for APS at the same time for 73 patients from the total of 106 patients included. We evaluated a large spectrum of APLAs: IgG and IgM anticardiolipin antibodies (aCL Abs), IgG and IgM anti-β2 glycoprotein I antibodies (aβ2GPI Abs), IgG and IgM antiphosphatidylserine antibodies (aPS Abs), IgG and IgM anti phosphatidylethanolamine antibodies (aPE Abs), IgG and IgM antiprothrombin antibodies (aPT Abs). We utilized for APLAs determinations the enzyme-linked immunosorbent assay (ELISA) kit from Aesku Diagnostics, Wendelsheim, Germany and the analyzer Chemwell 2910, Awareness Technology, Palm City, Florida, USA. The manufacturer limits for normal values are at less than 12U/ml, for equivocal values between 12 and 18U/ml, and positive values are considered superior to 18U/ml. At inclusion, we did not have the technical support for the reevaluation of the lupus anticoagulant (LAC); the data for LAC were registered retrospectively when more than two positives determinations were found in the patient's file, accordingly to classification criteria.

Statistical analysis

For the statistical analysis, SPSS 20.0 (IBM Corporation, USA) was used. For comparing the dichotomous nominal variables we used the non-parametric Chi-squared test. The risk was expressed by the estimate odds ratio (OR) together with 95% confidence interval (95% CI). For non-parametric con-

tinue variables, the results were expressed as median together with minimum, respectively maximum values. For parametric continues variables we calculate the mean and standard deviation (SD). The differences were considered significant when p-value was less than 0.05.

RESULTS

1. Study population

A total of 106 patients were enrolled in study, 36 patients diagnosed with primary APS and 70 patients with secondary APS. In the subgroup of patients with secondary APS, there were: a majority of 47 patients with SLE, 6 patients with rheumatoid arthritis, 4 patients with Sjögren's syndrome, 4 patients with Behçet's disease, 2 patients with systemic sclerosis, 2 patients with panarteritis nodosa, 2 patients with mixed connective tissue disease, 1 patients with inflammatory myositis, 1 patient with cancer, and 1 patients with chronic D hepatitis.

The majority of the patients in the study cohort were females, with a female-to-male ratio of 6.6. The mean age at inclusion was 44.7 years. The mean age at the moment of APS diagnosis was 38.0 years and the average disease duration of 6.7 years (Table 1).

Venous thromboses were the most frequent thrombotic events, found in 48 patients (45.3%). They were followed by arterial thrombosis and gestational morbidity (34.0%, respectively 25.0%). The distribution of the venous thromboses was dominated by the presence of lower limb DVT (35.8%). Combined thrombosis, venous and arterial, was found in only a minority of patients (6.6%) as presented in Table 1.

LAC was the most frequent biological parameter which diagnosed APS in our patients (96.2%). When the diagnosis of APS was sustained by the concomitant presence of two positive biological parameters, the most frequent combination were LAC and aCL Abs (19.8%), respectively LAC and aβ2GPI Abs (21.7%) (Table 1).

2. General features

The mean period of evolution for patients with secondary APS was longer than that of patients with primary APS (3.6 years vs 8.4 years, $p < 0.05$) (Table 2). No significant difference was found for the occurrence for positivity of APLAs in primary APS patients when compared to secondary APS patients (data not shown).

Our subgroup of patients with secondary APS was not homogenous; we noted a predominance of the patients with APS secondary to SLE. Because in our analyses we did not found differences between

TABLE 1. *Baseline characteristics of the entire study group (n = 106)*

Characteristic		
Sex F/M,(F%)		92/14 (86.8)
Age at inclusion, years mean±SD		44.7 ± 12.4
Age at APS's diagnosis, years mean±SD		38.0 ± 11.8
Disease duration, years mean±SD		6.7 ± 6.9
APS no(primary/secondary)		36/70
Venous thrombosis no (%)		48 (45.3)
DVT – upper extremities no (%)		7 (6.6)
DVT – lower extremities no (%)		38 (35.8)
Venous thrombosis in other territories (cave, porte, retina) no (%)		7 (6.6)
Superficial thrombosis no (%)		15 (16.7)
Recurrent venous thrombosis no (%)		14 (13.2)
Arterial thrombosis no (%)		36 (34.0)
Gestation pathology no (%)		23 (25.0)
Venous thrombosis and arterial thrombosis no (%)		8 (7.5)
Venous thrombosis and gestation pathology no (%)		3 (2.8)
Venous thrombosis, arterial thrombosis and gestation pathology no (%)		1 (0.9)
Positives LAC no (%)		102 (96.2)
Positives aCL Abs no (%)		23 (21.7)
Positives aβ2GPI Abs no (%)		23 (21.7)
Positives LAC + aCL Abs no (%)		21 (19.8)
Positives LAC + aβ2GPI Abs no (%)		23 (21.7)
Positives aCL Abs + aβ2GPI Abs no (%)		7 (6.6)
Positives LAC + aCL Abs + aβ2GPI Abs no(%)		7 (6.6)

TABLE 2. *General features of the patients with primary APS, respectively secondary APS*

Characteristic	Primary APS	Secondary APS
	n = 36	n = 70
Age at inclusion, years mean ± SD	43.3 ± 12.0	45.4 ± 12.6
Age at APS diagnosis, years mean ± SD	39.8 ± 11.1	37.1 ± 12.1
Disease duration, years mean ± SD	3.6 ± 3.2	8.4 ± 7.7*

*p < 0.05

the patients with APS secondary to SLE and the patients with APS secondary to other disease than SLE (data not shown), we considered together all the patients with secondary APS regardless of the associated diseases.

3. Traditional risk factors for DVT

The traditional risk factors for DVT (smoking, BMI, waist circumference, waist-to-hip ratio) were similar in patients with APS and DVT history and in patients without DVT (for the later, the diagnostic of APS being based on other thrombotic or obstetrical events) (Table 3).

4. APLAs relation with DVT history

From the 73 patients tested for all APLAs at inclusion, 31 were patients with previous DVT and 42 were without previous DVT. The presence of positives IgM aβ2GPI Abs was significantly associated with the history of DVT. There were no other significant differences for the others criteria and non-criteria APLAs tested, as show in Table 4.

The titers of the non-criteria APLAs, IgG aPE Abs and IgG aPT Abs, were significantly higher in patients with DVT history when compared with patients without DVT (Table 5).

TABLE 3. *Presence of traditional risk factors for DVT in patients with/without DVT (data for 106 patients)*

Characteristic	APS with DVT	APS without DVT	P	OR (95% CI)
	n = 48	n = 58		
Age at inclusion, years mean ± SD	45.4 ± 10.7	44.0 ± 13.6	NS	–
Age at APS diagnosis, years mean ± SD	38.0 ± 10.2	38.0 ± 13.1	NS	–
Disease duration, years med (min-max)	5.5 (0.0-33.0)	3.0 (0.0-29.0)	NS	–
Sex, no F/M	42/6	50/8	NS	0.89 (0.26-2.84)
Smoking, no Y/N	11/37	20/38	NS	0.56 (0.23-1.34)
BMI, kg/m^2	26.6 ± 5.1	26.3 ± 5.7	NS	–
Feminine sex: Waist circumference, cm mean ± SD	91.0 ± 15.2	87.9 ± 14.7	NS	–
Masculine sex: Waist circumference, cm mean ± SD	102.8 ± 13.3	100.6 ± 13.1	NS	–
Feminine sex: Waist-to-hip ratio	0.9 ± 0.1	0.8 ± 0.1	NS	–
Masculine sex: Waist-to-hip ratio	1.0 ± 0.1	0.9 ± 0.1	NS	–

TABLE 4. *Prevalence of positives APLAs in subgroups of APS (with/without DVT)*

	ASP with DVT	ASP without DVT		
Characteristic	**n=48**	**n=58**	**P**	**OR (95% CI)**
LAC Y/N†	47/1	55/3	NS	2.54 (0.26-68.83)
IgG aCL Abs Y/N	3/28	1/41	NS	4.39 (0.43-44.41)
IgM aCL Abs Y/N	2/29	1/41	NS	2.75 (0.23-31.89)
IgG aβ2GPI Abs Y/N	4/27	0/42	NS	13.90 (0.72-268.64)
IgM aβ2GPI Abs Y/N	8/23	2/40	0,02	6.95 (1.36-35.58)
IgG aPS Abs Y/N	4/27	1/41	NS	6.07 (0.64-57.51)
IgM aPS Abs Y/N	2/29	1/41	NS	2.82 (0.24-32.67)
IgG aPE Abs Y/N	1/30	1/41	NS	1.36 (0.08-22.73)
IgM aPE Abs Y/N	8/23	4/38	NS	3.30 (0.89-12.21)
IgG aPT Abs Y/N	2/29	0/42	NS	1.06 (0.95-1.16)
IgM aPT Abs Y/N	2/29	0/42	NS	1.06 (0.95-1.16)

†LAC is determined only at the diagnostic of APS diagnosis; ‡All the other APLAs are determined at the moment of inclusion in 73 patients.

TABLE 5. *The titers of APLAs in subgroups of APS's patients (with/without DVT)*

Characteristic	APS with DVT	APS without DVT	P
	med (min-max)	med (min-max)	
IgG aCL Abs	1.0 (0.0-121.0)	1.0 (0.0-20.0)	NS
IgM aCL Abs	4.0 (0.0-41.0)	3.0 (0.0-26.0)	NS
IgG aβ2GPI Abs	4.0 (0.0-79.0)	3.0 (0.0-12.0)	NS
IgM aβ2GPI Abs	6.0 (0.0-300.0)	5.0 (0.0-36.0)	NS
IgG aPS Abs	2.0 (1.0-130.0)	2.0 (0.0-40.0)	NS
IgM aPS Abs	5.0 (0.0-31.0)	3.0 (1.0-26.0)	NS
IgG aPE Abs	3.0 (0.0-151.0)	2.0 (0.0-20.0)	0.01
IgM aPE Abs	5.0 (0.0-31.0)	3.0 (1.0-26.0)	NS
IgG aPT Abs	5.0 (1.0-33.00)	3.0 (1.0-7.0)	0.01
IgM aPT Abs	4.0 (0.0-82.0)	3.0 (0.0-13.0)	NS

† APLAs were determined at the moment of inclusion in the study.

The analysis by ROC curve found that the best predictor for DVT occurrence is the titer of IgG aPE Abs (AUC 0.659; 95%CI 0.524-0.794), followed by the titers of IgG aPT Abs (AUC 0.652; 95%CI 0.516-0.787), IgM aβ2GPI Abs (AUC 0.627; 95%CI 0.492-0.763) and IgM aPE Abs (AUC 0.625; 95%CI 0.493-0.758) (Figure 1).

5. Traditional risk factors for DVT in patients with primary and secondary APS

As for the whole group, the analysis of traditional risk factors for DVT in patients with primary or secondary APS did not reveal significant associations with the history of DVT (Table 6).

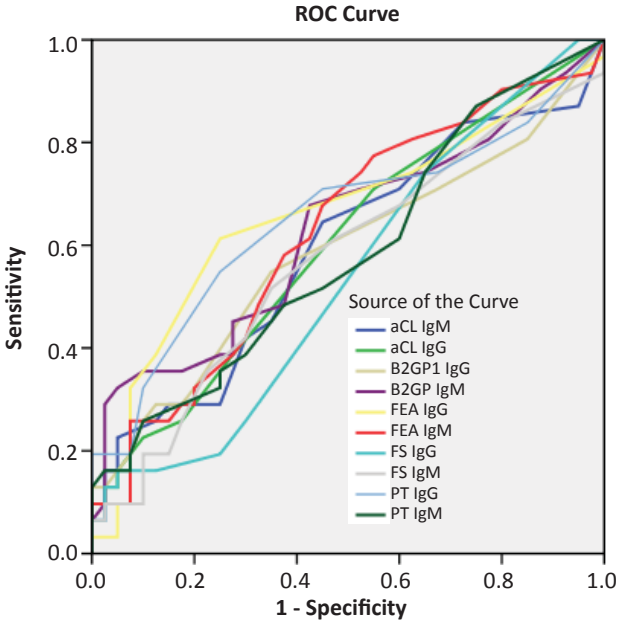

FIGURE 1. *ROC Curve for the titers of APLAs and the history of DVT (all patients)*

6. APLAs relation with DVT history in primary and secondary APS

In the subgroup of the primary APS, 30 patients had been tested for the APLAs at the inclusion; among them, 14 were patients with previous DVT. From the 43 patients with secondary APS which had been tested for the APLAs, 17 had a history of DVT.

None of the APLAs were found to be related to the DVT history in primary APS. For the secondary

TABLE 6. *Traditional risk factors for DVT in subgroups of patients with primary and secondary APS (with/without DVT)*

	Primary APS				Secondary APS			
	With DVT	Without DVT			With DVT	Without DVT		
	n = 18	n = 18			n = 30	n = 40		
Characteristic			**p**	**OR (95% CI)**			**p**	**OR (95% CI)**
Age at inclusion, years mean ± SD					45.6±11.5	45.2 ± 13.4	NS	–
Age at APS diagnosis, years mean ± SD	40.8 ± 8.4	38.8 ± 13.5	NS	-	36.4±10.9	37.7 ± 13.1	NS	–
Disease duration, years med (min-max)	4.2 ± 3.8	2.9 ± 2.5	NS	-	9.5±7.9	7.5 ± 7.4	NS	–
Sex F/M	16/2	14/4	NS	0.43 (0.06-2.76)	26/4	36/4	NS	1.38 (0.31-6.05)
Smoking Y/N	5/13	8/10	NS	0.48 (0.11-1.90)	6/24	12/28	NS	0.58 (0.19-1.79)
BMI, kg/m^2	26.1 ± 4.3	26.6 ± 5.6	NS	-	26.9 ± 5.6	26.2 ± 5.8	NS	–
Female: Waist circumference, cm mean ± SD	91.8 ± 11.9	87.0 ± 10.6	NS	-	90.6 ± 17.1	88.2 ± 16.2	NS	–
Male: Waist circumference, cm mean ± SD	88.0 ± 2.8	100 ± 14.3	NS	-	110.2 ± 8.6	101.2 ± 14.0	NS	–
Female: Waist-to-hip ratio	0.9 ± 0.1	0.8 ± 0.1	NS	-	0.9 ± 0.1	0.8 ± 0.1	NS	–
Male: Waist-to-hip ratio	0,95 ± 0,06	0,94 ± 0,08	NS		1,05 ± 0,14	0,96 ± 0,05	NS	

TABLE 7. *APLAs positivity in the subgroups of patients with primary and secondary APS (with/without DVT)*

	Primary APS				Secondary APS			
	With DVT	Without DVT			With DVT	Without DVT		
Characteristic			p	OR (95% CI)			p	OR (95% CI)
LAC Y/N	18/18	18/18	NS	1.00 (0.39-2.51)	29/1	37/3	NS	2.35 (0.23-23,80)
IgG aCL Abs Y/N	1/13	0/16	NS	3.66 (0.13-97.48)	2/15	1/25	NS	3.33 (0.27-39.97)
IgM aCL Abs Y/N	0/0	0/0	NS	1.00 (0.00-255.6)	2/15	1/25	NS	3.33 (0.27-39.97)
IgG aβ2GPI Abs Y/N	2/12	0/16	NS	6.60 (0.29-150.07)	2/15	0/26	NS	8.54 (0.38-189.82)
IgM aβ2GPI Abs Y/N	2/12	1/15	NS	2.50 (0.20-30.99)	6/11	1/25	0.02	13.63 (1.46-127.15)
IgG aPS Abs Y/N	1/13	0/16	NS	3.66 (0.13-97.48)	3/14	1/25	NS	5.35 (0.50-56.50)
IgM aPS Abs Y/N	0/0	0/0	NS	1.00 (0.00-255.6)	2/15	1/25	NS	3.33 (0.27-39.97)
IgG aPE Abs Y/N	0/14	1/15	NS	0.35 (0.01-9.46)	1/16	0/26	NS	4.81 (0.18-125.39)
IgM aPE Abs Y/N	2/10	0/16	NS	7.85 (0.34-180.33)	5/12	4/22	NS	2.29 (0.513-10.17)
IgG aPT Abs Y/N	1/13	0/16	NS	3.66 (0.13-97.48)	1/16	0/26	NS	4.81 (0.18-125.39)
IgM aPT Abs Y/N	1/13	0/16	NS	3.66 (0.13-97.48)	1/16	0/26	NS	4.81 (0.18-125.39)

†LAC is determined only at the time of APS diagnosis; ‡All the antibodies are determined at the moment of inclusion in the present study.

APS, only the IgM aβ2GPI Abs showed a significant higher prevalence in the subgroup of patients with previous DVT (Table 7).

When evaluating the titer of APLAs in patients with primary APS, only the IgG aPT Abs were significantly higher in patients with history of DVT (Table 8). These antibodies were the best predictors for the DVT history in patients with primary APS (AUC 0.743; 95%CI 0.557-0.929) (Figure 2).

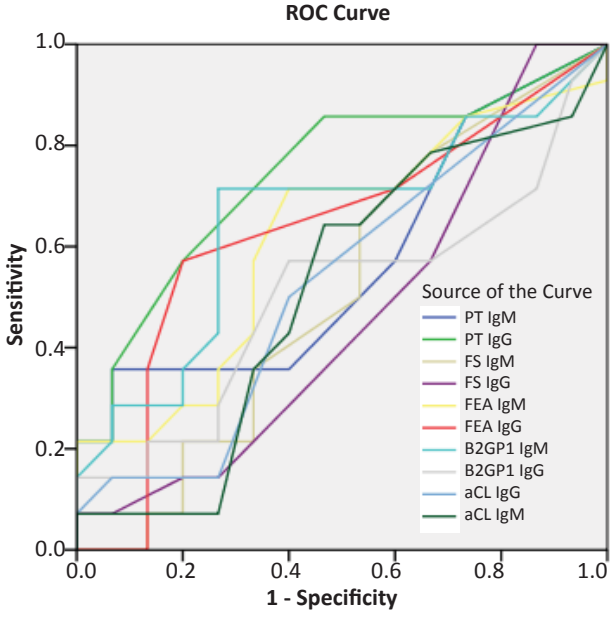

FIGURE 2. *ROC Curve for the titers of APLAs and the history of DVT (patients with primary APS)*

Concerning the secondary APS, the titer of IgG aCL Abs and also the titer of IgG aPE Abs were higher in the presence of previous DVT episode (Table 8). At ROC curve analysis, the higher AUC was obtained for the IgG aCL Abs (AUC 0.689; 95% 0.526-0.853) (Figure 3).

7. Factors associated with recurrence of DVT in patients with APS

From the 48 patients diagnosed with DVT (out of the total of 106 patients), a number of 13 patients presented recurrent episodes of DVT. From the total of 48 patients, 31 were tested for the APLAs at the inclusion; from them, 10 patients had previous recurrent DVT. The traditional risk factors for DVT (Table 9) and the prevalence of positive APLAs (Table 10) were similar in patients with or without DVT recurrences. However, the levels of IgG aPT Abs were higher in patients with DVT recurrences (Table 11).

DISCUSSIONS

The demographic data of the patients from our group (age of inclusion – 44.7 years, age at diagnosis – 38.0 years, with a mean disease duration of 6.7 years and a net predominance of female sex – 86.9%) are similar with those reported in the Euro-Phospholipid cohort (age of inclusion – 42.0 years, age at diagnosis – 34.0 years, with a mean disease duration of 7.6 years and 82.0% patients of female sex) (2).

For the mean disease duration, we found a significant longer disease evolution in patients with secondary APS when compared with those with primary APS (8.4 years versus 3.6 years). This difference might be explained by the fact that, during the evolution of the disease, primary APS becomes actually secondary APS by adding new features to the

TABLE 8. *Titers of APLAs in the subgroups of patients with primary and secondary APS (with/without DVT)*

	Primary APS			Secondary APS		
	With DVT	Without DVT		With DVT	Without DVT	
Characteristic	med (min-max)	med (min-max)	p	med (min-max)	med (min-max)	p
IgG aCL Abs	5.00 (0.00-121.00)	0.00 (0,00-4,00)	NS	2.00 (0.00-64.00)	1.00 (0.00-20.00)	0,02
IgM aCL Abs	4.00 (0.00-12.00)	3.50 (0.00-9.00)	NS	4.00 (0.00-41.00)	3.00 (0.00-26.00)	NS
IgG aβ2GPI Abs	4.00 (0.00-30.00)	3.00 (0.00-11.00)	NS	4.00 (0.00-79.00)	3.00 (0.00-12.00)	NS
IgM aβ2GPI Abs	6.00 (0.00-36.00)	4.50 (0.00-30.00)	NS	8.00 (0.00-300.00)	5.50 (0.00-36.00)	NS
IgG aPS Abs	2.00 (1.00-130.00)	2.00 (0.00-5.00)	NS	2.00 (1.00-112.00)	2.00 (1.00-40.00)	NS
IgM aPS Abs	3.50 (1.00-12.00)	4.00 (1.00-14.00)	NS	6.00 (0.00-31.00)	3.00 (1.00-26.00)	NS
IgG aPE Abs	3.00 (1.00-15.00)	2.00 (1.00-20.00)	NS	3.00 (0.00-151.00)	2.00 (0.00-7.00)	0,03
IgM aPE Abs	10.00 (0.00-23.00)	5.50 (1.00-17.00)	NS	8.00 (0.00-202.00)	6.00 (0.00-58.00)	NS
IgG aPT Abs	5.00 (1.00-33.00)	3.00 (1.00-7.00)	0,01	5.00 (1.00-20.00)	3.00 (1.00-7.00)	NS
IgM aPT Abs	2.00 (0.00-82.00)	2.50 (0.00-12.00)	NS	5.00 (0.00-25.00)	3.00 (0.00-13.00)	NS

APLAs were determined at the moment of inclusion.

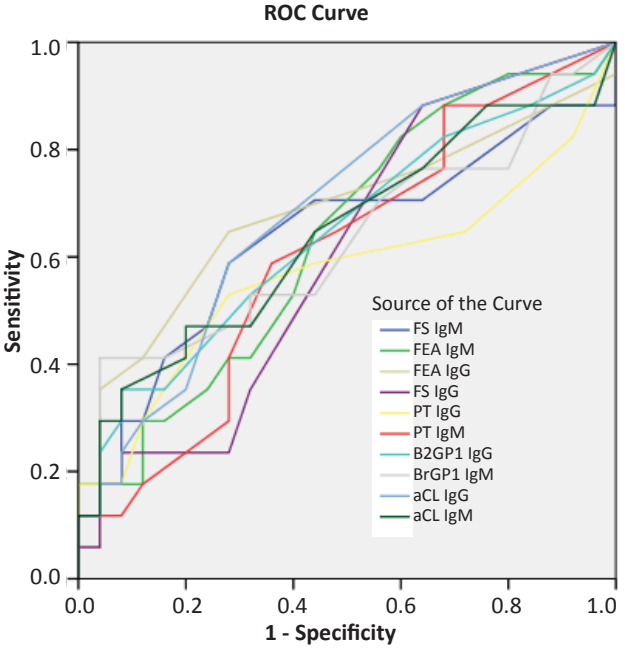

FIGURE 3. *ROC Curve for the titers of APLAs and the history of DVT (patients with secondary APS)*

initial clinical and immunological spectrum, especially clinical arguments for SLE (3,4).

In our study, the traditional risk factors for DVT were not fond to be related to the occurrence of DVT. That is why we suppose that there are other factors, possibly immunological, which favor the thrombotic events in patients with APS.

Over the past years there is an increasing interest for APS and for the APLAs, both criteria (aCL Abs, LAC, aβ2GPI Abs) and non-criteria (aPT Abs, aPE Abs, aPS Abs). Data from literature showed that the APLAs are found even in persons without thrombotic events. Unfortunately, the available APLAs tests cannot distinguish between the persons at risk of thrombosis and the patients which are not at risk (5).

Ruffatti et al published in 2011 a study that identified LAC as the only biological marker associated with the risk of venous thrombosis in APLAs carriers (6). Moreover, Ruffatti noted that LAC was an independent risk factor for the first thrombotic event in this particular population.

TABLE 9. *Characteristics, including traditional risk factors for DVT, in subgroups of patients with ASP (with/without recurrent DVT)*

	APS with recurrent DVT	APS without recurrent DVT		
Characteristic			**p**	**OR (95% CI)**
Age at inclusion, years mean ± SD	45.8±12.1	45.3±10.4	NS*	–
Age at APS diagnosis, years mean ± SD	39.4±9.4	37.9±12.2	NS	–
Disease duration, years med (min-max)	5.5(0-33)	3(0-29)	NS	–
Sex no (F/M)	3/10	3/32	NS	3.20 (0.55 -18.42)
Smoking no (Y/N)	3/10	8/27	NS	1.01 (0.22 – 4.59)
BMI , kg/m^2	26.5±5.1	26.7±5.2	NS	–
Feminine sex: Waist circumference, cm mean ± SD	93.7±14.9	88.7±14.9	NS	–
Masculine sex: Waist circumference, cm mean ± SD	102.0±13.9	101.5±13.1	NS	–
Feminine sex: Waist/hip ratio	0.9±0.1	0.9±0.1	NS	–
Masculine sex: Waist/hip ratio	1.0±0.1	0.9±0.1	NS	–

*NS = non-significant.

TABLE 10. *APLAs positivity in subgroups of patients with APS (with/without recurrent DVT)*

	APS with recurrent DVT	APS without recurrent DVT		
Characteristic			p	OR (95% CI)
LAC Y/N	12/1	35/0	NS	0.11 (0.00-3.07)
IgG aCL Abs Y/N	1/9	2/19	NS	1.05 (0.08-13.22)
IgM aCL Abs Y/N	0/10	2/19	NS	0.37 (0.01-8.47)
IgG aβ2GPI Abs Y/N	0/10	4/17	NS	0.18 (0.00-3.79)
IgM aβ2GPI Abs Y/N	0/10	8/13	NS	0.07 (0.00-1.46)
IgG aPS Abs Y/N	1/9	3/18	NS	0.66 (0.06-7.35)
IgM aPS Abs Y/N	0/10	2/19	NS	0.37 (0.01-8.47)
IgG aPE Abs Y/N	0/10	1/20	NS	0.65 (0.02-17.39)
IgM aPE Abs Y/N	1/9	7/14	NS	0.22 (0.02-2.12)
IgG aPT Abs Y/N	0/10	2/19	NS	0.37 (0.01-8.47)
IgM aPT Abs Y/N	0/10	2/19	NS	0.37 (0.01-8.47)

NS = non-significant (p-value > 0.05) ; †LAC is determined only on APS diagnosis;
‡All the other Abs are determined at the moment of inclusion

TABLE 11. *Titer of APLAs in the subgroups of APS's patients (with/without recurrent DVT)*

	APS with recurrent DVT	APS without Recurrent DVT	
Characteristic	med (min-max)	med (min-max)	p
IgG aCL Abs	0.0 (0.0-121.0)	1.0 (0.0-64.0)	NS*
IgM aCL Abs	4.0 (0.0-17.0)	4.0 (0.0-41.0)	NS
IgG aβ2GPI Abs	3.0 (0.0-8.0)	3.0 (0.0-79.0)	NS
IgM aβ2GPI Abs	4.0 (0.0-17.0)	6.0 (0.0-300.0)	NS
IgG aPS Abs	2.0 (1.0-130.0)	2.0 (0.0-112.0)	NS
IgM aPS Abs	3.0 (0.0-12.0)	4.0 (0.0-31.0)	NS
IgG aPE Abs	2.0 (0.0-15.0)	2.0 (0.0-151.0)	NS
IgM aPE Abs	7.0 (0.0-21.0)	8.0 (0.0-202.0)	NS
IgG aPT Abs	5.0 (1.0-33.0)	3.0 (1.0-7.0)	0.01
IgM aPT Abs	5.0 (0.0-14.0)	3.0 (0.0-82.0)	NS

*NS = non-significant; † APLAs are determined at the moment of inclusion in the present study

Eschwege et al found that LAC was the only laboratory parameter associated with the history of severe thrombosis (defined as recurrent thrombosis with early onset) (7). LAC was also noted as independent risk factor for first thrombotic event in APLAs carriers (6).

In the Euro-Lupus cohort, the LAC was found as the most frequent laboratory parameter (8) and apparently it is also the only one for which it was proved an association with the risk of thrombosis in SLE patients (9).

In patients with secondary APS, the presence of LAC or polyclonal aCL Abs is associated with risk of venous thrombosis, but it is LAC that determines a higher risk of thrombosis (10). On the contrary, in general population, the aCL Abs do not seem to increase the risk of venous thromboembolism (11).

A systematic review that assessed the relation between LAC and aCL Abs and the risk of thrombosis found that, in patients with APS, LAC is a risk factor for thrombosis (independent of the type of thrombosis, site of thrombosis, presence of SLE diagnosis or tests used for determination), while aCL Abs can identify the patients at risk of thrombosis only at the medium to high titer (12). Apparently, the presence of aCL Abs alone increases the risk of thrombosis with 4.7 to 5.5-fold (13). When compared with aβ2GPI Abs, the aCL Abs in medium to high titer are associated with the same risk of thrombosis (14). The results of different studies regarding the APLAs are not always concordant and this might be due to the lack of standardization for the tests used for detection (15,16).

Until now, two APS's Abs has been shown to have LAC activity: aβ2GPI Abs and aPT Abs. That demonstrates also that the LAC activity is not induced by phospholipids only (17). Initially, it was observed the LAC activity is frequently depending

of the presence of aβ2GPI Abs (18,19) but newer data showed that not all the aβ2GPI Abs have actually LAC activity. Apparently, only those aβ2GPI Abs that recognized the epitope Gly40-Arg43 in domain I of β2GPI have a LAC activity. And so, at least two different types of aβ2GPI Abs might be described depending on LAC activity (20).

In our study group, the LAC was the most frequent biological diagnostic parameter that has been found positive in the history of patients with both primary and secondary APS; it was practically positive in all patients with primary APS recruited for this study. Moreover we found that the presence of IgM aβ2GPI Abs was significantly associated with the history of DVT. These results are applied for the subgroup of patients with secondary APS where only the IgM aβ2GPI Abs showed a significantly higher prevalence in the lot of patients with previous DVT, but not for the subgroup with primary APS.

In general population, the presence of diagnostic LAC, aβ2GPI Abs and non-criteria aPT Abs was accompanied by an increase risk of a first DVT episode (21); the presence of aβ2GPI Abs alone seem to give an increase of DVT risk of 1.6-2.4-fold (13).

Some of the aPT Abs was shown to display prothrombinase activity and might play a catalytic role for thrombosis in APS (22). Probably, the aPT Abs activate the endothelial cell by increasing the expression adhesion molecules from E-selective class (23). Also, the IgG and IgM aPT Abs were found to be associated with primary APS and the IgG aPT Abs might be associated with the subset of patients with thrombotic events when compared with those with obstetrical events (24). Also, aPT Abs were proved to be correlated with the prevalence of thrombotic event in primary APS as well as in SLE patients (25). Similar to aβ2GPI Abs, the aPT Abs can be classified according to their different LAC activity (26). The observation that not all the serums positives for aPT Abs have LAC activity has led to the hypothesis of presence of two different types of aPT Abs, a functional one, that display also LAC activity and a non-functional one, that not exert a LAC activity (27). Even so, the aPT Abs with LAC activity seems to be less frequent associated with thrombosis than aβ2GPI Abs with intrinsic LAC activity (28).

In APS secondary to SLE, IgG aPT Abs were found to be correlated with thrombosis and, on the contrary, no correlation with thrombosis was found for the IgM aPT Abs. For the anti-prothrombin in complex with phosphatidylserine Abs (aPS/PT Abs), both IgM and IgG subtypes were correlated with thrombosis (29). The aPT Abs and anti-prothrombin in complex with phosphatidylserine Abs (aPS/PT Abs) can be one of the antigenic targets for lupus anticoagulant (30). Moreover, Zanon et al showed that aPT Abs might be involved in recurrent thromboembolism pathogenesis (31).

The observation that there are patients which present a clinical spectrum suggestive of APS but with negatives serum diagnostic APS Abs has led to the term "sero-negative" APS, an entity where other antigenic targets might be actives (32). Among the non-criteria Abs there are the aPT Abs, aPE Abs and aPS Abs.

Concerning the non-criteria aPE Abs, it was observed that there was a correlation with the presence of thrombosis but not with the APS diagnosis (33). In patients with SLE, the aPE Abs presence seems not to be associated with the risk of thrombosis (34). In other study, the aPE Abs were related with the presence of thrombosis (15% of patients with thrombosis were aPE Abs positives compared with 3% of controls); also, aPE Abs were positives in patients with thrombosis but without APS's diagnosis (negative LAC, aCL Abs or aβ2GPI Abs) (35). The mechanism of pro-thrombotic effects of aPE Abs is not known, but possibly the interference with the kininogen complex after biding the phosphatidiletanolamine may determine platelet aggregation (36).

A recent systematic review that included 30 studies with patients without SLE concluded that not only the criteria APS Abs (LAC, aCL Abs and aβ2GPI Abs) are related with risk of arterial thrombosis but also the non-criteria aPT Abs and aPS Abs. On the contrary, for venous thrombosis, only the classical LAC and aCL Abs were associated with an increased risk. There are not sufficient data for a clear conclusion regarding the role of aPE Abs in the pathogenesis of thrombosis (37).

Interesting, in our research we found that the titer for the non-criteria APS's Abs, IgG aPE Abs and IgG aPT Abs, was significantly higher in patients with DVT history compared with those without DVT in the clinical spectrum of their APS. Also, we found a significant greater titer for the IgG aPT Abs in the subgroup of patients with recurrent DVT. In general, the recurrent DVT was more frequently found in patients with primary APS than secondary APS. These results are concordant with previous published studies. The aPT Abs might have a role in the pathogenesis of DVT, probably expressed by their LAC activ-

ity. The clinical relevance of aPT Abs needs to be established by further prospective studies.

CONCLUSIONS

The results of our study showed an association between DVT and the presence of criteria IgM aβ2GPI Abs, and also with the titer of some non-criteria APS's Abs, IgG aPT Abs and IgG aPE Abs. For the patients with primary APS, the DVT was related only with the titer of IgG aPT Abs. Also, the recurrent DVT was associated with the presence of IgG aPT Abs. Future studies will probably prove the utility of aPT Abs as a diagnostic tool in APS, maybe by defining a certain APS subgroup with negatives classic APLAs.

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