Association between systemic non-criteria APS manifestations and antibody type and level: results from the Serbian national cohort study

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Abstract

Objectives

The aim of this study was to investigate the importance of aPL type and level for non-criteria-related events in APS patients.

Methods

Our study included 374 patients: 260 with PAPS and 114 with APS associated with systemic lupus erythematosus (SLE).

Results

We discovered significant connection between migraine and LA absence, livedo reticularis and aCL-IgG, skin ulcerations with aCL-IgG and anti-β2GPI-IgM, pseudovasculitis lesions with aCL-IgG, aCL-IgM and anti-β2GPI-IgM, and thrombocytopenia with aCL-IgM, aCL-IgG and anti-β2GPI-IgG. Thrombocytopenia occurred more frequently in patients with more than one aPL. In PAPS, epilepsy correlated with ß2GPI-IgM, migraine with aCL-IgM, and thrombocytopenia with aCL-IgM, aCL-IgG, anti ß2GPI-IgG and LA. Skin ulcerations occurred more frequently in IIc category patients and in patients with high levels of aCL-IgG and anti ß2GPI-IgG. Livedo reticularis was more prominent in PAPS with high levels of aCL-IgG. Significantly higher prevalence of thrombocytopenia was observed in patients with high levels of aCL-IgG and anti ß2GPI-IgG. Epilepsy was related to high levels of anti ß2GPI-IgM and thrombocytopenia in the SAPS was correlated with aCL-IgG. Skin ulcerations were more prevalent in aCL-IgM positive SAPS patients and epilepsy more frequently in SAPS patients with high levels of anti ß2GPI-IgG.

Conclusion

Our study showed that certain aPL type with certain level correlated with non-criteria manifestations, suggesting their predictive role.

Key words

antiphospholipid antibody type and level, antiphospholipid syndrome, non-criteria manifestations, Hughes syndrome

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Introduction

Antiphospholipid syndrome (APS), or Hughes syndrome, is traditionally described as a syndrome consisting of recurrent fetal loss, vascular thrombosis, and positive aPL levels. Nowadays, APS is increasingly recognised as a multisystem disease, of which clinical expression may include cardiac, neurological, haematological, cutaneous, and other manifestations (1-11).

The prevalence of the syndrome with no associated systemic connective tissue diseases (primary APS) in the general population is still a matter of debate, since there are no sound epidemiological studies in the literature so far (12). aPL displays higher prevalence in systemic lupus erythematosus and rheumatoid arthritis than in other systemic autoimmune diseases. However, not all the aPL positive lupus patients display clinical manifestations.

Different types and levels of aPL appear to be important for the frequency of non-criteria APS manifestations (13, 14).

Central nervous system involvement is one of the most prominent manifestations of APS; various non-criteria CNS manifestations such as dementia, epilepsy, migraine, cognitive dysfunctions, and chorea have also been associated with aPL (15).

Skin manifestations such as livedo reticularis, pseudovasculitis, and skin ulcerations, are less common in APS patients. On the other hand, skin involvement may be the first manifestation of APS in 40% of the patients, suggesting its predictive role in thrombotic event occurrence, and indicating the importance of testing for aPL in all subjects with skin disorders (16, 17).

Various haematological non-criteria manifestations have also been described in association with APS. Thrombocytopenia is frequently found in APS patients, with incidence ranging from 22–42% in different studies. It is usually moderate (>$50\times10^{9/L}$) and benign. However, it has shown to be associated with haemorrhage complications rarely. But commonly it is without clinical manifestation, thus requiring no intervention (18).

The diagnosis of seronegative APS has been suggested for patients with clinical manifestations indicative of APS, but with persistently negative results in the commonly used assays to detect anti-cardiolipin (aCL) antibodies, anti-β2 glycoprotein I antibodies (aβ2GPI), and lupus anticoagulant (LA) (19).

APS is associated with a variety of cardiac abnormalities. In the most recent consensus conference in Sydney, Australia, valvular heart disease was accepted as an integral part of the syndrome (20).

Patients and methods

Patients

We analysed 374 APS patients (Caucasians): 260 PAPS (69.5%, 76.2% female and 23.8% male) patients with average age 45.60±13.33 years and 114 (30.5%) SAPS (SLE) patients (87.7% female and 12.3% male) of average age 46.29±15.01 years. Patients with APS have been included, consecutively, starting from the year 2000 to date in the prospective manner and registered as the Serbian National Cohort Study. We investigated the association between non-criteria manifestations and aPL type and its level. All patients with SLE met the American College of Rheumatology (ACR) classification criteria (2). All patients analysed have met the 2006 revised Sydney criteria for APS, suggesting that all patients were diagnosed with APS not only by the presence of antiphospholipid antibodies, but also according to other diagnostic criteria (arterial and/or venous thrombosis, multiple and recurrent fetal losses) (20). However, aPL analysis was performed routinely in SLE patients. The patients were not included in the study unless they met the 2006 revised Sydney criteria for APS and even if the patients were positive aPL finding. Besides thrombotic manifestations, systemic non-criteria manifestations were also observed in APS patients.

The study follows the ethical guidelines of the most recent Declaration of Helsinki (Edinburgh, 2000) and has received approval from the local ethical committee. All patients were examined by council consisting of rheumatologist, neurologist, ophthalmologist, psychiatrist, pulmonologist, cardiologist, radiologist, dermatologist, and haematologist.

Diagnosis of non-criteria APS manifestations

Central nervous system manifestations. Central nervous system (CNS) non-criteria manifestations were defined as follows (19). Epilepsy was defined as at least two separate episodes of unprovoked seizures with an interval greater than 24h. Seizures could be either partial or generalised tonic-clonic. The seizures had to be unrelated to metabolic abnormalities and to drug or medication use or withdrawal. Additionally, seizures should have been occurred without other provoking factors such as hypoxia or cerebral hypoperfusion. Migraines were characterised as recurrent severe unilateral or bilateral headaches (lasting from 4 to72 hours, with or without aura), after excluding other relevant etiologies of these disorders such as tension headache, hypertension, or subacute endocarditis. Chorea was defined as an abnormal involuntary movement disorder, and characterised by brief irregular contractions that are not repetitive or rhythmic. However, chorea appears to flow from one muscle to the next, after excluding drugs, metabolic influence, endocrine disorders, and vascular incidents as common causes. In addition to neurologic and psychiatric examination, the CNS involvement was assessed with digital electroencephalography (Galileo System, Italy), visual, somatosensory, and acoustic evoked potentials ("Medilog-Sensor", Vickers Co.), electromyoneurography, and MRI (Gyroscan, Philips T5-NT). Initial scout MRI (performed on cross-sectional, frontal and sagittal planes) was followed by the assessment of cross-sectional planes by T-1 and T-2 relaxation. The following parameters were measured: signals from cerebral and cerebellar parenchyma, ventricles, basal cistern, and subarachnoid spaces of brain convexity. Results were obtained after consensus among three neuroradiologists who were double-blinded. Neuropsychological testing involved Folstein's mini-mental test, standardised Wechsler adult intelligence scale test, Boston naming test, figure drawing test, speech fluency, computerised Cambridge neuropsychological test automated battery (CANTAB) test, finger-tapping test, and Purdue's Pegboard test. Cognitive function was assessed according to five established categories: 1) general IQ (assessed from total, verbal and non-verbal IQ); 2) speech (assessed by Boston naming test and correct responses with and without semantic support); 3) attention (assessed by tests of total, verbal and non-verbal summary); 4) memory (assessed by tests of visual, spatial, and complex memory, recognition time, visual-associative memory and its time span); 5) executive functions, which reflect "frontal" cognitive functions such as decision making, planning, and problem solving.

Standard reference values were used for comparison purposes in the assessments of IQ, speech and executive functions. The final diagnosis was based on consensus of several medical specialists.

Skin manifestations. Livedo reticularis, skin ulcerations and pseudovasculitis were diagnosed by a rheumatologist and a dermatologist. Skin manifestations were also confirmed by a skin biopsy, when it was necessary for differential diagnosis of cutaneous involvement of APS.

Haematological manifestations. Thrombocytopenia was defined as platelet count equal or less than $100x10^9$ platelets/l on at least two separate occasions. Low platelet count in patients treated with heparin, low-molecular weight heparin, or immunosuppressive drugs was not considered as a positive criterion for thrombocytopenia.

Cardiac manifestations.

All enrolled patients underwent transthoracic echocardiogram. Transthoracic echocardiography was performed using a standardised protocol that included M-mode, 2-dimensional (2-D), and Doppler recordings. Valvular lesions were defined as focal leaflet thickening, which were unlikely to represent age-related valvular thickening. Severity of valvular regurgitation was characterised using standard criteria (20). The diagnosis of non-stable angina pectoris was established according to the presence of chest pain (with or without ST segment), T wave ECG alterations, and the absence of elevated I troponin levels.

Patient selection

All diagnostic procedures were carried out at the time of diagnosis of APS. Patients were assessed at two to six monthly intervals, according to standard protocol, which included a complete history of patients with their physical and laboratory examinations.

APS patients were classified into the following categories: category I, where two or more aPL are present together, category IIa, where lupus anticoagulant is present alone, category IIb, where only anti-cardiolipin antibodies (aCL) are present, and category IIc, where anti-β2 glycoprotein-I antibodies (anti-β2GPI) are present (14).

Laboratory tests

All patients were evaluated for the presence of antiphospholipid antibodies, accompanied by routine biochemistry tests and complete blood cell counts. Lupus anticoagulant (LA) was based on the initial use of phospholipid-depleted or platelet-depleted coagulation tests such as kaolin clotting time (KCT), dilute Russell's venom viper time (DRVVT), the tissue thromboplastin inhibition test and diluted activated partial thromboplastin time 19. The LA tests were not performed while the patients were receiving anticoagulant therapy. Anti-cardiolipin (aCL: IgG/IgM) and anti-β2glycoprotein I (β2GPI: IgG/IgM) antibodies were measured by an enzyme-linked immunosorbent assay (ELISA, Binding Site) and were expressed in GPL or phospholipid (MPL) units (GPL-U and MPL-U). The level range was considered by positive levels as low (11–40 PLU/ml), medium (41–99 PLU/ml), and high (>100PLU/ml). Also, we monitored revised laboratory criteria for APS on two or more occasions at least 12 weeks apart (8). Antinuclear antibodies (ANA) were determined by indirect immunofluorescence on mouse liver and HEp-2 cell substrate. Anti-double-stranded DNA (anti-dsDNA) antibodies were determined by ELISA binding site.

Statistical analysis

The χ^2 and Fisher's exact tests were done, as needed, to analyse statistically significant differences between cat-

egorical variables. Two-sided probability (*p*-) values of less than 0.05 were considered significant. Analysis was performed with the SPSS statistical package Version 14.0 (SPSS, Chicago, IL, USA).

Results

The prevalence and localisation of non-criteria manifestations

Neurological, skin, and haematological non-criteria manifestations were evaluated in all patients. The most frequent manifestation in PAPS group was migraine 70 (26.9%), while in SAPS group it was livedo reticularis 76 (66.7%). Chorea was observed only in SAPS patients. Epilepsy (p=0.0001), livedo reticularis (p=0.0001), pseudovasculitis (p=0.0001), skin ulcerations (p=0.0001), and thrombocytopenia (p=0.0001) were observed significantly more frequently in patients with SAPS (Table I). Surprisingly, prevalence of migraine as well as non-stable angina pectoris, valve thickening, and dysfunction did not differ significantly between SAPS and PAPS patients (p=0.167, p=0.564, and p=0.182, respectively).

Table I. Prevalence of non-criteria manifestations in patients with primary and secondary APS.

Non-criteria manifestations	n (%) patients with primary APS (n=260)	n (%) patients with secondary APS (n=114)	*p*-value
Epilepsy	5 (8.4)	21 (18.4)	p=0.0001
Chorea	0 (0)	9 (7.9)	p=0.0001
Migraine	70 (26.9)	37 (32.5)	p=0.167
Livedo reticularis	34 (13.1)	76 (66.7)	p=0.0001
Pseudovasculitis	33 (12.7)	68 (59.6)	p=0.0001
Skin ulcerations	25 (9.6)	40 (35.1)	p=0.0001
Thrombocytopenia	43 (16.5)	44 (38.6)	p=0.0001
Non-stable angina pectoris	25 (9.6)	11 (9.6)	p=0.564
Valve thickening and dysfunction	9 (3.5)	7 (6.1)	p=0.182

Table II. Distribution of aPL in the PAPS and SAPS groups.

	n (%) patients with primary APS (n=260)	n (%) patients with secondary APS (n=114)	*p*-value
aPL type			
aCL IgG	95 (36.5)	68 (59.6)	0.0001
aCL IgM	141 (54.2)	73 (64.0)	0.049
β2GPI IgG	83 (31.9)	49 (43.0)	0.027
β2GPI IgM	98 (37.7)	51 (44.7)	0.122
LA	133 (51.2)	56 (49.1)	0.402
aPL category			
I	160 (61.5)	81 (71.1)	
IIa	41 (15.8)	5 (4.4)	
IIb	46 (17.7)	23 (20.2)	p=0.020
IIc	13 (5.0)	5 (4.4)	

PAPS: primary antiphospholipid syndrome; SAPS: secondary antiphospholipid syndrome; aCL: anticardiolipin antibodies; β2 GPI: anti- β2 glycoprotein I antibodies; LA: lupus anticoagulant; aPL: antiphospholipid antibodies.
Categories: I-more than one aPL present, IIa LA present alone, IIb-aCL present alone, IIc- anti-β2GPI present alone.

Distribution of patients according to antibody categories

The distribution of aPL in the PAPS and SLE groups revealed high statistical significance in presence of aCL IgG and IgM, and β2GPI IgG antibodies, as presented in the Table II. More than one type of antibodies (category I) was present in 241 patients (category I). In contrast, 160 (66.4%) were PAPS patients and 81 (33.6%) were SLE patients. Lupus anticoagulant was present in only 46 (12.3%) patients (category IIa). The aCL antibodies were present in 69 (18.4%) patients (category IIb). Anti-β2GPI antibodies were present in only 18 (4.8%) patients (category IIc) (Table II).

Association of non-criteria manifestations with aPL type and level

The prevalence of analysed non-criteria manifestations was similar in all antibody category groups, except for thrombocytopenia, which occurred more frequently in patients with more than one aPL present (p=0.008) (Fig. 1). Statistical analysis showed significant connection between migraine occurrence and LA absence (p=0.014). Significant positive connection was revealed between livedo reticularis and aCL-IgG (p=0.002), skin ulcerations with aCL-IgG (p=0.025) and anti-β2GPI IgM positively (p=0.018), pseudovasculitis lesions with aCL IgG (p=0.002), aCL IgM (p=0.020) and anti-β2GPI IgM positively (p=0.025), and thrombocytopenia with aCL-IgM (p=0.047), aCL-IgG (p=0.0001), anti-β2GPI IgG (p=0.013) presence in all patients analysed. Regarding cardiac manifestations, valve thickening and dysfunction occurrence was significantly related to LA presence (p=0.039) and anti β2 GPI IgG positively (p=0.035) (Table III).

The levels of various types of antibodies also correlated with the prevalence of specific non-criteria manifestation. Livedo reticularis occurred more frequently in patients with high levels of aCL IgG (p=0.041), pseudovasculitis occurred in patients with medium levels of anti β2GPI IgM (p=0.043), thrombocytopenia appeared in APS patients with high levels of aCL IgG (p=0.0001) and anti β2GPI IgG (p=0.004), epilepsy in patients with high levels of anti β2GPI IgG (p=0.010) and anti β2GPI IgM (p=0.001), and chorea occurred in patients with medium levels of anti β2GPI IgM (p=0.010). Valvular changes occurred with higher prevalence in patients with high levels of aCL IgG present (p=0.015) (Table III).

Non-criteria manifestations in PAPS

In the PAPS patients, epilepsy correlated with the presence of β2GPI IgM antibodies (p=0.019), migraine with presence of aCL IgM (p=0.017), and thrombocytopenia with presence of aCL IgM (p=0.004), aCL IgG (p=0.018), anti β2GPI IgG (p=0.046) and LA (p=0.032).

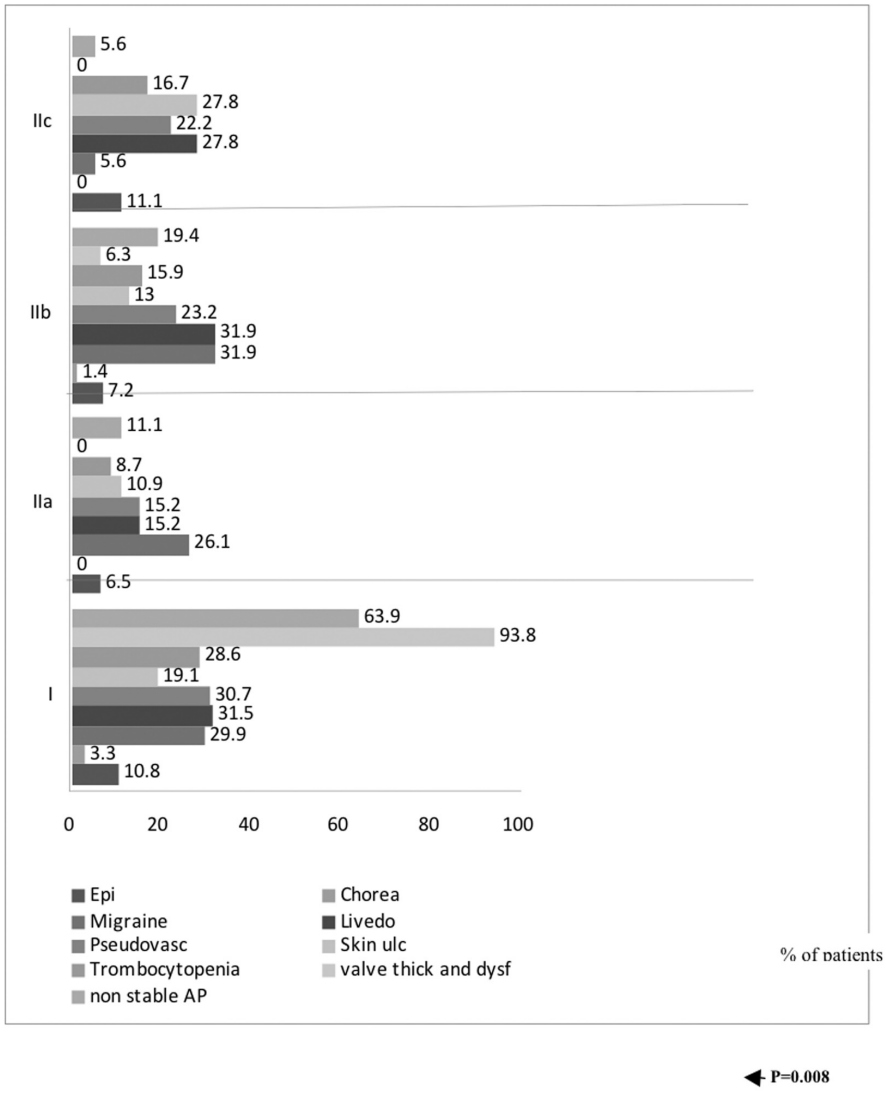

Fig. 1. Distribution of non-criteria manifestations in different categories of APS patients.

Skin ulcerations occurred more frequently in IIc category patients (p=0.048) (Fig. 2) who had high levels of aCL IgG (p=0.022) and anti β2GPI IgG (p=0.041). Livedo reticularis was more prominent in PAPS patients with high levels of aCL IgG (p=0.0001). Significantly higher prevalence of thrombocytopenia was noticed in patients with high levels of aCL IgG (p=0.001) and anti β2GPI IgG (p=0.001). Moreover, in our study, epilepsy was related to high levels of anti β2GPI IgM (p=0.0001). Although the analysed cardiac manifestations were not related to specific type of aPL in PAPS patients, statistical analysis revealed significant correlation between non-stable angina pectoris and medium levels of anti β2 GPI IgG (p=0.017). Valvulopathies occurred more frequently in patients with high levels of anti β2 GPI IgM (p=0.022) (Table IV).

Non-criteria manifestations in SAPS

Despite the observations given in PAPS patients, thrombocytopenia in the SAPS group correlated only with aCL IgG presence (p=0.007). Skin ulcerations were more prevalent in aCL IgM positive SAPS patients (p=0.007), and valvular changes in anti β2 GPI IgM positive patients (p=0.027). (Table V)

There was no correlation between different categories of SAPS patients and non-criteria manifestations.

As presented in Table V, correlation between non-criteria manifestations and levels of aPL present in SAPS patients was observed with less prominence than in PAPS patients. The patients with epilepsy had high levels of anti β2GPI IgG present (p=0.017) more frequently, and patients with valvular changes were more likely to have high levels of aCL IgG (p=0.013) and anti β2 GPI IgG (p=0.036) antibodies.

Discussion

This study assessed correlations between non-criteria APS manifestations and various types and levels of aPL antibodies. Asherson *et al*. reported that non-thrombotic clinical features were relatively common in APS patients, and included thrombocytopenia, livedo reticularis, haemolytic anaemia, epilepsy, leg ulcers, amaurosis fugax, chorea, cutaneous necrosis, etc., with prevalence lower than 5% (23, 24). Another study performed on 90 Israeli APS patients showed no correlation between antibody levels (LA, IgG aCL, IgM aCL) and clinical manifestations (27). However, other papers confirmed that LA positively (OR: 3.753) seemed to be a prognostic marker for non-criteria APS manifestations (28).

According to Liu *et al*., SLE patients with central nervous system involvement have significantly higher levels of aCL IgG and aCL IgM (28). Recent studies have shown that neurological APS manifestations were associated with high levels of aCL IgG (>100GPL) (mostly in relatively young patients) and often associated with tobacco abuse, hyperlipidaemia, LA, systemic ischaemic events, and occult cardiac disease (29-33). These results were obtained in only 27 patients.

In our results, only epilepsy correlated with medium levels of aCL IgG. We also showed a correlation between medium levels of both aCL and β2GPI IgG/IgM antibodies, and CNS manifestations such as epilepsy, dementia, and chorea. In our patients, chorea was diagnosed only in the SAPS patients, which showed a significant correlation with medium levels of aCL IgM and β2GPI IgG/IgM. This is inconsistent with the data in the literature, where the absence of aCL IgM in patients with APS supports prior evidence that aCL IgG and LA may be more clinically relevant antibodies for thrombosis.

Table III. Distribution of non-criteria manifestations regarding aPL type and level in all patients analysed.

Non-thrombotic manifestation	n (%) of APS patients		
	aPL type		
	Positive	Negative	*p*-value
	LA		
Migraine	44 (23.3)	63 (34.1)	*p*=0.014
Valve thick and dys	12 (6.3)	4 (2.2)	*p*=0.039
	aCL IgG		
Livedo reticularis	61 (37.4)	49 (23.2)	*p*=0.002
Pseudovasculitis	57 (35.0)	44 (20.9)	*p*=0.002
Skin ulcerations	36 (22.1)	29 (13.7)	*p*=0.025
Thrombocytopenia	57 (35.0)	30 (14.2)	*p*=0.0001
	aCL IgM		
Pseudovasculitis	67 (31.1)	34 (21.3)	*p*=0.020
Thrombocytopenia	57 (26.6)	30 (18.8)	*p*=0.047
	β2 GPI IgG		
Valve thick and dys	11 (68.8)	152 (42.5)	*p*=0.035
Thrombocytopenia	40 (30.3)	47 (19.4)	*p*=0.013
	β2 GPI IgM		
Pseudovasculitis	49 (32.9)	52 (23.1)	*p*=0.025
Skin ulcerations	34 (22.8)	31 (13.8)	*p*=0.018

	aPL level			
	Low	Medium	High	*p*-value
	aCL IgG			
Livedo reticularis	87 (27.3)	10 (34.5)	13 (50.0)	*p*=0.041
Thrombocytopenia	67 (21.0)	5 (17.2)	15 (57.7)	*p*=0.0001
Valve thick and dys	11 (3.4)	1 (3.4)	4 (15.4)	*p*=0.015
	β2 GPI IgG			
Epilepsy	29 (8.6)	1 (2.8)	6 (31.6)	*p*=0.004
Thrombocytopenia	74 (21.9)	3 (17.6)	10 (52.6)	*p*=0.007
	β2 GPI IgM			
Chorea	6 (1.8)	2 (13.3)	1 (5.9)	*p*=0.010
Epilepsy	28 (8.2)	2 (13.3)	6 (35.3)	*p*=0.001
Pseudovasculitis	87 (25.4)	8 (53.3)	6 (35.3)	*p*=0.043

aCL: anticardiolipin antibodies; β2 GPI: anti- β2 glycoprotein I antibodies; LA: lupus anticoagulant; aPL: antiphospholipid antibodies.

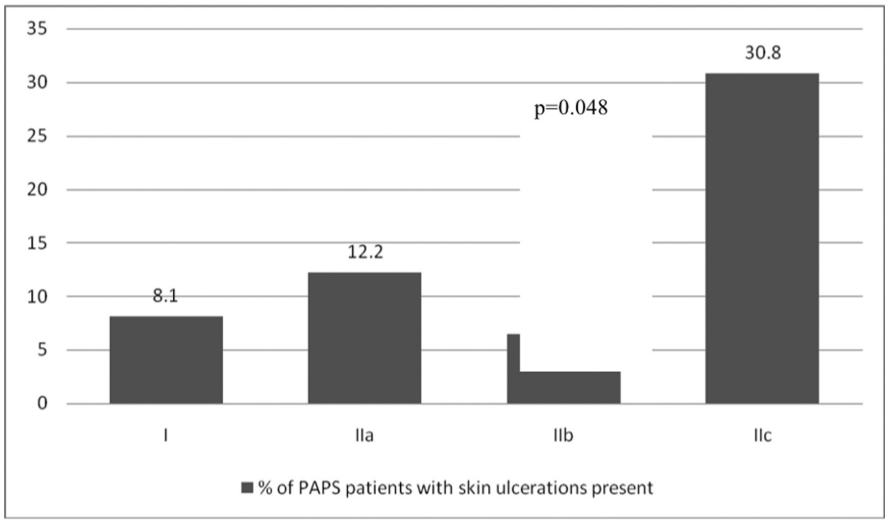

Fig. 2. Different categories of aPL and skin ulcerations in PAPS patients.

However, according to Orzechowski *et al*., aCL IgM may be important in patients with chorea (34). Some reports indicate high prevalence of aPL levels in chorea patients, but with no explanation of cut-off values and types of aPL antibodies tested (34, 35). With respect to CNS events, possible mechanisms include vascular occlusion and injury by pathogenic aPL antibodies in a disrupted blood-brain barrier (36, 37).

It is indicated that patients with APS have deteriorated endothelium-dependent and independent vascular function which could be, together with increased inflammatory response, involved in vascular complications in these patients (38, 39).

Previous findings showed that an increased prevalence of livedo reticularis, cardiac valve disease, and cognitive dysfunction co-occurred with high levels of aCL. These manifestations were also more common in combination with high levels of β2GPI (>80 IU/ml), with respect to different cut-off values, which is mostly consistent with our results (40, 41). In our study, patients with high levels of aCL IgM and β2GPI IgM (>100 IU/ml) were more likely to develop skin manifestations. We also confirmed correlations between epilepsy, dementia, chorea and medium levels of both aCL and β2GPI, but there are no data in the literature revealing that problem.

Skin lesions were less common and had previously been reported as part of Sneddon's syndrome, an uncommon disorder which is characterised by multiple cerebrovascular accidents (24%), along with idiopathic livedo reticularis (44%) and systemic APS appearances (32%). It was previously reported that 75% of SAPS patients had classic symptoms, while 25% of the patients had systemic APS signs without livedo reticularis (42, 43). No correlation was shown between livedo reticularis and the presence of positive aPL antibodies (44, 45). Two patients were described to have extensive cutaneous necrosis associated with high levels of aCL antibodies (46). Our results confirmed correlations between skin ulcerations, pseudovasculitis, and livedo reticularis with high levels of

Table IV. Distribution of non-criteria manifestations regarding aPL type and level in PAPS patients.

Non-thrombotic manifestation	n (%) of PAPS patients		
	aPL type		
	Positive	Negative	*p*-value
	LA		
Migraine	28 (21.1)	42 (33.1)	*p*=0.020
Thrombocytopenia	28 (21.1)	15 (11.8)	*p*=0.032
	aCL IgG		
Thrombocytopenia	24 (25.3)	19 (11.5)	*p*=0.004
	aCL IgM		
Migraine	46 (32.6)	24 (20.2)	*p*=0.017
Thrombocytopenia	30 (21.3)	13 (10.9)	*p*=0.018
	β2 GPI IgG		
Thrombocytopenia	19 (22.9)	24 (13.6)	*p*=0.046
	β2 GPI IgM		
Epilepsy	10 (10.2)	5 (3.1)	*p*=0.019
	aPL level		
	Low	Medium	High	*p*-value
	aCL IgG			
Livedo reticularis	25 (10.9)	2 (5.9)	7 (50.0)	*p*=0.0001
Skin ulcerations	21 (9.2)	0 (0)	4 (28.6)	*p*=0.022
Thrombocytopenia	34 (14.8)	1 (5.9)	8 (57.1)	*p*=0.0001
	β2 GPI IgG			
Skin ulcerations	20 (8.5)	1 (7.7)	4 (33.3)	*p*=0.017
Thrombocytopenia	35 (14.9)	2 (15.4)	6 (50.0)	*p*=0.006
Non-stable angina pectoris	21 (8.9)	4 (30.8)	0 (0.0)	*p*=0.017
	β2 GPI IgM			
Epilepsy	9 (3.7)	1 (14.3)	5 (45.5)	*p*=0.0001
Valve thick and dys	7 (2.9)	0 (0.0)	2 (18.2)	*p*=0.022

PAPS: primary antiphospholipid syndrome; aCL: anticardiolipin antibodies; β2 GPI: anti- β2 glycoprotein I antibodies; LA: lupus anticoagulant; aPL: antiphospholipid antibodies.

Table V. Distribution of non-criteria manifestations regarding aPL type and level in SAPS patients.

Non-thrombotic manifestation	n (%) of SAPS patients		
	aPL type		
	Positive	Negative	*p*-value
	aCL IgG		
Thrombocytopenia	33 (48.5)	11 (23.9)	*p*=0.007
	aCL IgM		
Skin ulcerations	32 (43.8)	8 (19.5)	*p*=0.007
	β2 GPI IgM		
Valve thickness and dysfunction	6 (12.0)	1 (1.6)	*p*=0.027
	aPL level		
	Low	Medium	High	*p*-value
	aCL IgG			
Valve thickness and dysfunction	3 (3.3)	1 (8.3)	3 (25.0)	*p*=0.013
	β2 GPI IgG			
Epilepsy	17 (16.5)	0 (0.0)	4 (57.1)	*p*=0.017
Valve thickness and dysfunction	5 (4.9)	0 (0.0)	2 (28.6)	*p*=0.036

SAPS: secondary antiphospholipid syndrome; aCL: anticardiolipin antibodies; β2 GPI: anti- β2 glycoprotein I antibodies; LA: lupus anticoagulant; aPL: antiphospholipid antibodies.

aCL IgM and β2GPI IgM ($p<0.005$). Definite mechanisms have not yet been established (48, 49).

Thrombocytopenia was significantly more common in the SAPS patients (39.4%), which is consistent with the literature, where the prevalence was 29.3% (50). The majority of our patients had high levels of aCL IgG and β2GPI IgG. However, a few studies have considered the correlation between thrombocytopenia and the levels of aPL, indicating the need for further evaluation. Thrombocytopenia in APS has been attributed to the presence of antibodies, directed against platelet glycoproteins, which appear to have different epitopes than aPL. Exposure of the inner layer platelet membrane of phospholipids can lead to an interaction with aPL, which may in turn contribute to lower platelet counts as well as to other systemic manifestations of APS (50, 51). Low aPL antibody levels did not correlate with any of the assessed clinical manifestations in our patients, which is consistent with recent reports (52, 53).

Non-criteria manifestations were frequent in APS patients and correlated with particular aPL. We found that thrombocytopenia was more common in PAPS and SAPS patients with high levels of aCL IgG and β2GPI IgG/IgM. Chorea was manifested only in the SLE patients, which correlated with medium levels of aCL IgM and β2GPI IgM. Patients in both groups with high levels of aCL IgM and β2GPI IgM were more prone to skin disorders.

The general conclusion is that there are significantly more non-criteria clinical manifestations in SAPS then in PAPS, probably in accordance to the associated autoimmune disease. Finally, after a 10-year follow-up, we noticed that some patients with high level of aPL, beside non-criteria manifestations, seem to never develop any thrombotic manifestation of APS.

The increased awareness of the role of humoral immunophysiology in APS has aroused interest in B cells as therapeutic targets in this disease. Future randomised controlled clinical trials will determine if B cell depletors and/or B cell modulators can be effective agents for treating patients with APS (54).

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