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 β2GPI-IgM, migraine with aCL-IgM, and thrombocytopenia with aCL-IgM, aCL-IgG and anti-β2GPI-IgG. Skin ulcerations occurred more frequently in IIc category patients and in patients with high levels of aCL-IgG and anti-β2GPI-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-β2GPI-IgG. Epilepsy was related to high levels of anti-β2GPI-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-β2GPI-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

Antiphospholipid antibody and non-criteria manifestations / L. Stojanovich et al.

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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 x 10^9/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.

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Antiphospholipid antibody and non-criteria manifestations / L. Stojanovich et al.

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 to 72 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.), electromyoneuropathy, 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); 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 100x10⁹ 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 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 χ² 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).

Distribution of patients according to antibody category
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=0.0002), 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 β2GPI IgG positively (p=0.035) (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).

<table>
<thead>
<tr>
<th>Table I. Prevalence of non-criteria manifestations in patients with primary and secondary APS.</th>
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<tbody>
<tr>
<td>Non-criteria manifestations</td>
</tr>
<tr>
<td>---------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Epilepsy</td>
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<tr>
<td>Chorea</td>
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<tr>
<td>Migraine</td>
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<tr>
<td>Livedo reticularis</td>
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<tr>
<td>Pseudovasculitis</td>
</tr>
<tr>
<td>Skin ulcerations</td>
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<tr>
<td>Thrombocytopenia</td>
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<tr>
<td>Non-stable angina pectoris</td>
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<tr>
<td>Valve thickening and dysfunction</td>
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<table>
<thead>
<tr>
<th>Table II. Distribution of aPL in the PAPS and SAPS groups.</th>
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<tbody>
<tr>
<td>aPL type</td>
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<tr>
<td>-------------------------------------------------------------</td>
</tr>
<tr>
<td>aCL IgG</td>
</tr>
<tr>
<td>aCL IgM</td>
</tr>
<tr>
<td>β2GPI IgG</td>
</tr>
<tr>
<td>β2GPI IgM</td>
</tr>
<tr>
<td>LA</td>
</tr>
</tbody>
</table>


Categories: I-more than one aPL present, IIa LA present alone, IIb-aCL present alone, IIc- anti-β2GPI present alone.
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-\(\beta_2\)GPI IgG \((p=0.041)\). Livedo reticularis was more prominent in patients with high levels of aCL IgG \((p=0.001)\). Significantly higher prevalence of thrombocytopenia was noticed in patients with high levels of aCL IgG \((p=0.001)\) and anti-\(\beta_2\)GPI IgG \((p=0.001)\). Moreover, in our study, epilepsy was related to high levels of anti-\(\beta_2\)GPI IgM \((p=0.0001)\). Although the analyses 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-\(\beta_2\)GPI IgG \((p=0.017)\). Valvulopathies occurred more frequently in patients with high levels of anti-\(\beta_2\)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-\(\beta_2\)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-\(\beta_2\)GPI 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-\(\beta_2\)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 \((>100\text{GPL})\) (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 anti-\(\beta_2\)GPI 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 anti-\(\beta_2\)GPI 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.
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

![Graph](image)

**Fig. 2.** Different categories of aPL and skin ulcerations in PAPS patients.
Antiphospholipid antibody and non-criteria manifestations / L. Stojanovich et al.

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

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


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

<table>
<thead>
<tr>
<th>Non-thrombotic manifestation</th>
<th>n (%) of SAPS patients</th>
<th>aPL type</th>
<th></th>
<th></th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>33 (48.5)</td>
<td>11 (23.9)</td>
<td></td>
<td></td>
<td>0.007</td>
</tr>
<tr>
<td>Skin ulcerations</td>
<td>32 (43.8)</td>
<td>8 (19.5)</td>
<td></td>
<td></td>
<td>0.007</td>
</tr>
<tr>
<td>Valve thickness and dysfunction</td>
<td>6 (12.0)</td>
<td>1 (1.6)</td>
<td></td>
<td></td>
<td>0.027</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>aPL level</th>
<th>Low</th>
<th>Medium</th>
<th>High</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valve thickness and dysfunction</td>
<td>3 (3.3)</td>
<td>1 (8.3)</td>
<td>3 (25.0)</td>
<td>0.013</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>17 (16.5)</td>
<td>0 (0.0)</td>
<td>4 (57.1)</td>
<td>0.017</td>
</tr>
<tr>
<td>Valve thickness and dysfunction</td>
<td>5 (4.9)</td>
<td>0 (0.0)</td>
<td>2 (28.6)</td>
<td>0.036</td>
</tr>
</tbody>
</table>


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 IgM. 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).
Antiphospholipid antibody and non-criteria manifestations / L. Stojanovich et al.

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