Non-thrombotic Neurological and Cardiac Manifestations:
In a Cohort of Serbian Patients with Primary and Secondary Antiphospholipid Syndrome

Ljudmila Stojanovich, Aleksandra Djokovic, Milica Kontic, Dusica Smiljanic, Nenad Ilijevski, Vladimir Bojovic, Dragomir Marisavljevic

Abstract—Central nervous system involvement is one of the most prominent clinical manifestations of antiphospholipid syndrome (APS), and includes thrombotic events, psychiatric features and a variety of other non-thrombotic neurological syndromes. The aim of our prospective study was to investigate relationship between non-thrombotic neurological and cardiac manifestations. We analyzed 218 patients with primary (PAPS) and 115 patients with secondary APS (SAPS). Chorea, migraine and epilepsy occurred more frequently in SAPS, while headache and depression were more common in PAPS. Patients from both subgroups with unstable angina pectoris were more prone to TIA, epilepsy and transient global amnesia in PAPS and acute ischemic encephalopathy in SAPS. Patients with valve vegetations were more prone to epilepsy and depression. We revealed positive relationship between serum aCL IgG levels and incidence of acute ischemic encephalopathy in SAPS, aCL IgM and epilepsy in SAPS, aCL IgM and migraine in PAPS, β2GPI IgG and chorea in SAPS and β2GPI IgM and TIA and epilepsy in PAPS. LA was linked to more frequent occurrence of depression, transient global amnesia, and migraine in PAPS. In summary, we revealed strong link between some non-thrombotic neurological and cardiac manifestations in APS patients, underlying complexity and evolutionary nature of APS which highlights the outstanding need for multidisciplinary approach to treatment of these patients.

Keywords—antiphospholipid syndrome; non-thrombotic neurological manifestations; non-thrombotic cardiac manifestations.

I. INTRODUCTION

Antiphospholipid syndrome (APS) or Huges Syndrome represents a systemic autoimmune disorder characterized by arterial and/or venous thrombosis, multiple and recurrent fetal losses. APS is often accompanied by a thrombocytopenia and elevated levels of antiphospholipid antibodies (aPL), such as lupus anticoagulant (LA), anticardiolipin antibodies (aCL) and anti-β2GPI antibodies [1, 2, 3]. This syndrome is considered primary (PAPS) if unassociated with any other connective tissue disease or secondary (SAPS) if it appears in relationship with other autoimmune disorders, mainly systemic lupus erythematosus (SLE) [4-13].

Both in PAPS and SAPS neurological and psychiatric manifestations are common. They include stroke, transient ischemic attack (TIA), epilepsy, dementia, cognitive deficits, headaches, psychiatric disorders, seizures, chorea, MS-like manifestations, myelopathy, transverse myelitis, and ocular symptoms [4]. Many of these manifestations are interrelated and may occur simultaneously in the same patient. Neurological disorders may result from vascular thrombotic events or without thrombotic events - by direct injury to neuronal tissue [7]. This spectrum of neurological and psychiatric disorders in patients with antiphospholipid antibodies is sometimes referred as the “neuropsychiatric APS”.

II. MATERIALS AND METHODS

We examined 218 patients with PAPS (166 female and 52 male, mean age 45.8±13.7 years), and 115 SAPS patients, of which 108 were also diagnosed with SLE (100 female and 8 male, mean age 46.9±15.8 years) and 7 patients with Sjogren syndrome (6 female and 1 male, mean age 50.1±15.9 years). Among these groups were 14 patients with catastrophic APS (CAPS), which are in international registry created in 2000 by the European Forum on Antiphospholipid Antibodies [14,15].
Our study involved consecutive APS patients from 2000 to 2011. Patients included in the study met the 1997 revised Sapporo criteria for APS [3]. All patients with SLE met the American College of Rheumatology (ACR) classification criteria [16]. Disease activity was assessed by SLEDAI score at the time of enrollment [17]. The study met ethical guidelines of the Helsinki declaration (Edinburgh, 2000) and has received approval from the local ethical committee. All patients were examined by a council comprised of rheumatologist, neurologist, neuro-ophthalmologist, psychiatrist, pulmonologist, cardiologist, radiologist and hematologist.

A. Assessment of neurological manifestations

Diagnosis of neurological manifestations was established by clinical findings of transient or permanent focal neurological sign (such as hemiparesis, hemihypestesia, speech disorder, diplopia, defects of vision field) or presence of behavioral changes, migraine headache, seizures, movement disorders, sign of encephalopathy, cognitive decline, dementia.

Additionally, diagnostic process was followed by electroencephalography (EEG). We performed routine EEG examination, which involved >20 min 16-channel recording and activation methods HV 1 IFS. We noted focal, lateralized or generalized epileptic discharges, irritative changes, and slow activities. Nuclear magnetic resonance (NMR) of endoceranium (and in some cases of medulla spinalis) with T1W, T2W, and Flair sequences revealed focal or multifocal lacunar or macroischemic lesions, multiple sclerosis-like findings, and diffuse leuencephalopathy.

B. Neuropsychological testing

Neuropsychological testing involved Folstein's mini-mental test, the standardized Wechsler adult intelligence scale, the Boston naming test, the figure drawing test, speech fluency, the computerized Cambridge neuropsychological test automated battery (CANTAB), the finger-tapping test, and the Purdue pegboard test. Cognitive function was assessed according to five established categories: a) General IQ, assessed from total, verbal and non-verbal IQ; b) Speech, assessed by the Boston naming test and correct responses with and without semantic support; c) Attention, assessed by tests of total, verbal and non-verbal summation; d) Memory, assessed by tests of visual, spatial and complex memory, recognition time, visual-associative memory and its time span; and e) 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. Cognitive and memory function were compared to the performance of 50 control patients who were comparable in age, but showed no signs or symptoms of systemic disease.

C. Cardiac manifestations

The diagnosis of non-stable angina pectoris was established according to presence of chest pain, with or without ST segment and T wave ECG alterations and absence of elevated I troponin levels. In the setting of high troponin I levels with chest pain and/or ECG changes (e.g., ST segment elevation or denivelation) we diagnosed acute myocardial infarction. Heart failure (HF) was defined as inability of the heart to supply sufficient blood flow to meet the body's needs presenting as shortness of breath (typically worse when lying flat, which is called orthopnea), coughing, chronic venous congestion, ankle swelling, and exercise intolerance. Acute heart failure was diagnosed if the symptoms occurred promptly, whereas chronic heart failure was defined in patients having constant need for cardiac therapy.

All patients underwent transthoracic echocardiogram. Transthoracic echocardiography was performed using a standardized protocol that included M-mode, 2-dimensional (2-D), and Doppler recordings. Valvular lesions were defined as focal leaflet thickening, unlikely representing age-related valvular thickening. Severity of valvular regurgitation was characterized using standard criteria [18]. Presence of valve vegetations was defined as deposition of small sterile vegetations on valve leaflets histological made of fibrin (eosinophilic) and platelets, but devoid of PMNs, microorganisms, or inflammation.

D. Serological tests

All patients were evaluated for the presence of antiphospholipid antibodies with routine biochemistry 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. The LA tests were not performed while the patients were receiving anticoagulant therapy. Anticardiolipin (aCL: IgG/IgM) and anti-ß-glycoprotein I (ß-gpI: IgG/IgM) antibodies were measured by an enzyme-linked immunosorbent assay (ELISA, Binding Site) and expressed in GPL or phospholipids (MPL) units (GPL-U and MPL-U). Also, we followed revised laboratory criteria for APS [19]:

- Lupus anticoagulant present in plasma, on two or more occasions at least 12 weeks apart, detected according to the guidelines of the International Society on Thrombosis and Hemostasis (Scientific Subcommittee on lupus anticoagulant/phospholipid-dependent antibodies).
- Anticardiolipin antibody of IgG or IgM isotype, or both, in serum or plasma, present in medium or high titres (ie, >40 GPL or MPL, or greater than the 99th percentile) on two or more occasions, at least 12 weeks apart, measured by a standardised ELISA.
- Anti-ß2-glycoprotein 1 antibody of IgG or IgM isotype, or both, in serum or plasma (in titres greater than the 99th percentile), present on two or more occasions, at least 12 weeks apart, measured by a standardised ELISA

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.

E. Statistics

Results are shown as the mean ± SD, except for frequencies,
which are expressed as percentages. Comparisons between the
groups were made with chi-square analysis for categorical
variables with Yates or Pearson correction for continuity if
necessary. Two sided probability (p) values <0.05 (2-tailed)
were considered significant. All statistics analysis was
performed with using SPSS 16.0 statistical package.

III. RESULTS

Distribution of aPL antibodies reveals that aCL IgG were more

<table>
<thead>
<tr>
<th>aPL</th>
<th>PAPS n=218</th>
<th>SAPS n=115</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>aCL IgG</td>
<td>78 (35.8%)</td>
<td>62 (53.9%)</td>
<td>0.001</td>
</tr>
<tr>
<td>aCL IgM</td>
<td>118 (54.1%)</td>
<td>75 (65.2%)</td>
<td>0.096</td>
</tr>
<tr>
<td>β2GPI IgG</td>
<td>76 (34.9%)</td>
<td>52 (45.2%)</td>
<td>0.239</td>
</tr>
<tr>
<td>β2GPI IgM</td>
<td>75 (34.4%)</td>
<td>54 (46.6%)</td>
<td>0.092</td>
</tr>
<tr>
<td>LA</td>
<td>122 (51.4%)</td>
<td>52 (45.2%)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Legend: PAPS= primary antiphospholipid syndrome, SAPS= secondary
antiphospholipid syndrome, aCL= anticardiolipin antibodies, β2GPI=antι- 82
glycoprotein I antibodies, LA= lupus anticoagulant, aPL= antiphospholipid
antibodies

common in patients with SAPS (p=0.001, Table 1) and
presence of LA was significantly more common (p=0.002) in
patients with primary antiphospholipid syndrome (PAPS). There was no statistically significant relationship between the
presences of other aPL antibodies. In addition, no relationship
between gender and non-thrombotic neurological
manifestations in PAPS or SAPS was observed.

Chorea (p<0.001), migraine (p=0.026) and epilepsy (p=0.001)
ocurred more often in patients with SAPS, while headache
(p=0.031) and depression (p=0.037) were more common in
PAPS patients. Other non-thrombotic neurological
manifestations occurred in both groups of patients without any
significant correlation (Table 2).

Furthermore, we analyzed relationship between presence of
aPL antibodies with non-thrombotic neurological
manifestations in both PAPS and SAPS group. Our study
revealed statistical significance considering the presence of
aCL IgG and acute ischemic encephalopathy (p=0.035) in
SAPS group, aCL IgM and epilepsy (p=0.030) in SAPS group,
aCL IgM and migraine (p=0.016) in PAPS, β2GPI IgG and
chorea (p=0.041) in SAPS, β2GPI IgM and transient ischemic
attack (p=0.018) and epilepsy (p=0.036) in PAPS and the
presence of LA and depression (p=0.012), transient global
amnesia (p=0.049) and migraine (p=0.036) in PAPS group.

We also analyzed relationship between non-thrombotic
neurological manifestations and non-thrombotic cardiac
manifestations in both PAPS and SAPS group (Table 3 and 4).
Patients who had unstable angina pectoris were more likely to
develop transient ischemic attack in both PAPS (p=0.002) and
SAPS group (p=0.004), epilepsy (p=0.006) and transient
global amnesia (p=0.002) in PAPS and acute ischemic
encephalopathy (p=0.001) in SAPS. Patients with valve
vegetations were more prone to epilepsy (p=0.001) and
depression (p=0.002) in PAPS.
### TABLE III
THE RELATIONSHIP BETWEEN NON-TROMBOTIC NEUROLOGICAL AND CARDIAC MANIFESTATIONS IN PAPS GROUP

<table>
<thead>
<tr>
<th></th>
<th>Transient ischemic attack (N)</th>
<th>Epilepsy (N)</th>
<th>Transient global amnesia (N)</th>
<th>Depression (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>not present</td>
<td>present</td>
<td>p</td>
<td>not present</td>
</tr>
<tr>
<td>Non stable angina pectoris</td>
<td>not present</td>
<td>158</td>
<td>36</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>present</td>
<td>13</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Valve vegetations</td>
<td>not present</td>
<td>160</td>
<td>40</td>
<td>0.064</td>
</tr>
<tr>
<td></td>
<td>present</td>
<td>11</td>
<td>7</td>
<td></td>
</tr>
</tbody>
</table>

Legend: PAPS= primary antiphospholipid syndrome, SAPS= secondary antiphospholipid syndrome

### TABLE IV
THE RELATIONSHIP BETWEEN NON-TROMBOTIC NEUROLOGICAL AND CARDIAC MANIFESTATIONS IN SAPS GROUP

<table>
<thead>
<tr>
<th></th>
<th>Transient ischemic attack (N)</th>
<th>Acute ischemic encephalopathy (N)</th>
<th>Vertigo (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>not present</td>
<td>present</td>
<td>p</td>
</tr>
<tr>
<td>Non stable angina pectoris</td>
<td>not present</td>
<td>80</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>present</td>
<td>4</td>
<td>7</td>
</tr>
</tbody>
</table>

Legend: PAPS= primary antiphospholipid syndrome, SAPS= secondary antiphospholipid syndrome
Finally, no relationship between age, smoking habits or disease duration and non-thrombotic neurological manifestations in PAPS or SAPS group was found.

IV. DISCUSSION

We present the first results from our prospective study with APS patients, which started in 2000, and performed in University Medical Center, “Bezanijska Kosa”, Belgrade, in collaboration with other Clinical Centers in Serbia. Beside frequent clinical manifestations of antiphospholipid syndrome, we followed less common, unusual and rare manifestations, according to Ruiz-Irastorza and colleagues [14].

This is our first, national study that examines the relationship between antiphospholipid antibody type and level on non-thrombotic neurological manifestations, as well as association between non-thrombotic neurological and cardiac manifestations in both PAPS and SAPS group of patients. We observed many non-thrombotic neurological manifestations, both in PAPS and in SAPS patients, and determined that some of them occurred significantly more often than others in both groups of APS patients: chorea, migraine and epilepsy occurred more often in patients with SAPS, and headache and depression were more often in PAPS patients. Migraine and transient ischemic attack were the most common clinical findings in both groups, with total prevalence of 30.3% and 23.4% patients, respectively. The frequency of the most often neurological manifestations in our study group was comparable to data reported previously [4,7,19].

Our study revealed statistically significant difference between patients with primary and secondary APS with respect to the presence of specific aPL. aCL IgG antibodies were statistically more frequent in patients with secondary antiphospholipid syndrome (SAPS) and presence of LA was significantly more common in patients with primary antiphospholipid syndrome (PAPS). This study also indicates that presence of certain types and levels of aPL are associated with increased probability of some non-thrombotic neurological manifestations. We revealed significance considering the presence of aCL IgG and acute ischemic encephalopathy in SAPS group, between presence of aCL IgM and epilepsy in SAPS and aCL IgM and migraine in PAPS group. There was strong relationship between the presence of β2GPI IgG and chorea in SAPS, between β2GPI IgM and transient ischemic attack and epilepsy in PAPS and between LA and depression, transient global amnesia and migraine in PAPS.

Patients with unstable angina pectoris were more likely to develop transient ischemic attack in both PAPS and SAPS group, epilepsy and transient global amnesia in PAPS and acute ischemic encephalopathy in SAPS. Patients with valve vegetations were more prone to epilepsy and depression in PAPS. Krause and group have shown significant relationship between valvular heart disease and cerebrovascular manifestations - not only stroke and TIs, but also migraine and epilepsy [20]. Our study confirmed that presence of cardiac manifestations may be a risk factor for several types of CNS involvement in APS [21-37].

Relationship between gender, age and disease duration and neurological manifestations (both in PAPS and SAPS groups) has been rarely examined in previous studies. Our study showed that gender, age, smoking habits and disease duration were not associated with non-thrombotic neurological manifestations, both in PAPS and SAPS. In conclusion, we revealed strong link between some non-thrombotic neurological and cardiac manifestations in APS patients, underlying complexity and evolutionary nature of APS which highlights the outstanding need for multidisciplinary approach to treatment of these patients.

ACKNOWLEDGMENT

We thank all patients who participated in this study and our colleagues from many clinics in Serbia.

REFERENCES

[18] Zogbhi WA, Enriquez-Sarano M, Foster E, et al. Recommendations for evaluation of the severity of native valvular regurgitation with two-


