

PHD THESES

PERSONAL EXPERIENCES OBTAINED WITH FOLLOW-UP OF LUPUS PATIENTS WITH SPECIAL REGARDS TO THE IMPORTANCE OF ANTI- PHOSPHOLIPID ANTIBODIES

Tünde Tarr M.D.

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Tutor: Dr. Emese Kiss MD, PhD

Supervisor: Prof. Dr. Margit Zeher MD, DSci

3rd Department of Medicine, Institute for Medicine, Medical and Health Science Centre,

University of Debrecen

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INTRODUCTION

Antiphospholipid syndrome (APS) is characterised by recurrent pregnancy loss, thrombotic complications and the presence of anti-cardiolipin antibodies (aCL) and/or lupus anticoagulant (LAC) according to the Sapporo criteria. Antibody against β 2-glycoprotein I (a β 2GPI) has also been incorporated into classification criteria as revised in 2004 in Sydney. It may exist in its primary form, but may also be associated with variable disorders, especially systemic lupus erythematosus (SLE). LAC and aCL were included into the revised classification criteria of SLE as suggested by the American College of Rheumatology (ACR) in 1997. In recent years, there is better understanding regarding the features of antiphospholipid/ β 2GPI antibodies and their mechanism in thrombotic processes. Rare antiphospholipid antibodies such as anti-phosphatidyl-serine, anti-prothrombin, anti annexin V were also published. However, the predictive value of these antibodies in the development of thrombosis and the risk factors predisposing anti-phospholipid antibody (aPL) positive patients to clinical complications require further investigation. Despite publications on SLE with APS, only few prospective studies analysed the association between different antiphospholipid antibodies and the development of thrombosis in lupus patients. It has been shown that aPL may appear years before the onset of lupus. Recent publications have reported the occurrence of aPL antibodies prior to SLE diagnosis. Examination of pre-diagnosis autoantibody profile and its relation to clinical outcome can provide an insight to disease process, but with limited clinical significance. However, patients having a particular autoimmune disorder are recommended for screening of different autoantibodies and regular follow-ups for evaluation to other autoimmune disorders, overlapping or associating with the initial disease. Secondary APS as the manifestation of lupus is well characterised, but little is known about primary APS preceding and subsequently progressing into SLE.

Although studies on the evidence-based management of thrombosis in APS have been published, no clear guidelines are available regarding primary prophylaxis among antibody positive patients without thrombotic complications.

AIMS

Aims of the present work were:

- to analyse the risk factors predisposing to the development of clinical thrombotic events in lupus patients with and without antiphospholipid antibodies.
- to analyse thrombotic complications in patients with simple or multiple antiphospholipid antibodies.
- to analyse the development of new antiphospholipid antibody and thrombotic complications in antiphospholipid antibody positive lupus patients during a five year follow up.
- to determine the association between the presence and/or appearance of other, previously undetected, aPL with the clinical presentations of antiphospholipid syndrome.
- to collect all incident thrombosis- and antithrombotic therapy-related data at regularly scheduled visits.
- to characterise patients presenting with primary APS and later on progressed to SLE as regards their thrombotic events and SLE symptoms.
- to compare these data to those of matched groups of SLE patients without APS and to patients with secondary APS, which developed following SLE.
- to detect the rare phospholipid/cofactor autoantibodies in lupus patients.

PATIENTS AND METHODS

Patients

Lupus patients, who had been under regular medical supervision, were enrolled into the study. All met four or more of the revised American College of Rheumatology classification criteria for SLE. Antiphospholipid syndrome was diagnosed according to the Sapporo classification criteria and revised according to those defined in Sydney. Deep venous thrombosis, pulmonary embolism, myocardial infarction, TIA and stroke were diagnosed using ECG, appropriate laboratory (D-dimer, CK, LDH, Cardiac Troponin I) and imaging (Doppler sonography, ventilation/perfusion scintigraphy, angiography, thoracic echocardiography, CT, MRI) examinations as required. Regarding obstetrical outcomes adequate medical records were not available in all cases, and where relevant the type of foetal loss was characterized based on personal interviews. Antiphospholipid antibodies were determined at each visit. As blood sampling for the determination of different autoantibodies and immunologic parameters are a part of regular patient follow-up, no special ethical approval was required.

Methods

The IgG and IgM type beta2-glycoprotein-I dependent aCL antibody (positive above 22 GPL or 16 MPL units/ml) and antibody against beta2-GPI (positive above 14.6 SGU/ml or 34 U/ml for IgM) were measured by in house enzyme-linked immunosorbent assays (ELISA). The IgG and IgM type rare antiphospholipid antibodies and other autoantibodies (aDNA, aSm, aSSA, aSSB) also were measured by commercial ELISA kits.

LAC was determined by coagulation assays as per international recommendations (28). First the activated partial thromboplastin time (aPTT) was measured and prolongation over 10 seconds that was incorrigible upon addition of platelet-poor normal plasma was considered pathologic. The next step measured the dilute prothrombin time and if this was non-confirmatory hexagonal phospholipid test was performed. If the screening aPTT was not

prolonged a LAC sensitive aPTT was done, and further examinations performed as mentioned above. For those patients who were receiving cumarin/warfarin the dilute prothrombin time was omitted and the hexagonal phospholipid test was performed. Other coagulopathies were excluded.

Statistical analysis

Kolmogorov-Smirnov test was used to determine normalcy of data. Student's t test and Mann-Whitney U test were used for comparison of numeric parameters, where applicable. Chi-square test was used for analysing qualitative differences. Spearman's test was used for determining the correlation between anti-cardiolipin and anti-beta2-glycoprotein I antibodies. P value <0.05 was considered statistically significant. SPSS statistical software for Windows (version 13.0) was used for all statistical procedures.

RESULTS, NEW FINDINGS

I. Five-year follow-up of lupus patients with secondary antiphospholipid syndrome

1. Baseline findings

In 1999 a total of 272 lupus patients, who had been under regular medical supervision, were enrolled into the study, and *in about half of whom (84/165) the presence of aPL was associated with clinical thrombotic complication*. Antiphospholipid antibodies were present at anytime before study baseline in 61% of patients (165/272). At baseline, patients were assigned to three different groups based on their history of thrombotic events and aPL profile: an aPL- group with 107 aPL negative patients, an aPL+ group consisting of 81 aPL positive patients without clinical thrombosis and an antiphospholipid syndrome (APS) group where the 84 aPL+ patients met the Sapporo criteria. In the APS group, 80 from the 84 also met the updated Sydney classification criteria for definite APS.

Anticardiolipin antibody was the most frequent aPL and was present in 148 patients (89,7 %) with aPL, the second one was anti b2GPI antibody (in 76-patients). Lupus anticoagulant was the least frequent aPL and was present in 35 patients (21.2% of patients with aPL and 12.9% of study population) only. However, the prevalence of LAC was significantly higher in those with APS than in aPL positive patients without thrombotic manifestations.

Comparing the aPL+ and APS groups, nor the frequency neither the concentration of aCL and a β 2GPI antibodies' IgG and IgM isotypes showed statistically significant difference. *There was a significant and strong positive correlation between aCL and a β 2GPI antibodies for both isotypes.*

Patients may have different types of aPL in their sera concomitantly. Ten percent of antibody positive patients (17/165) had all the three aPL types in their sera. When patients having only aCL were compared to those presenting with all three types of aPL (n=17, including 13 women), *I found that the cumulative presence of antiphospholipid antibodies further increased the prevalence of clinical thrombotic events.* Although, statistical significance was found only with DVT.

The prevalence of deep vein thrombosis, pulmonary embolism, stroke, myocardial infarction were significant higher in patients with aPL than in aPL negative group, but pregnancy losses were no differences.

As the isotype of aPL antibodies may modify clinical manifestations, the prevalence of the different APS symptoms in the presence of IgG and IgM aCL were different. *Thrombotic clinical symptoms were more frequent in patients with the presence of IgG and IgG+IgM aCL than in those with IgM aCL.*

2. Follow-up observation

During the five years of follow-up, the baseline aPL profile changed in 15 patients. Eight of these 15 patients were aPL- at baseline. New clinical symptoms, characteristic for

APS were also registered during the five-year follow-up. From the aPL-, aPL+ and APS groups 2.8%, 3.7% and 8.3% of the patients had new or recurrent thrombotic complications, respectively. In the initially aPL- group, DVT occurred in one patient along with the appearance of aCL, CVA was observed in 2 patients together with new a β 2GPI, and one had severe thrombocytopenia along with the appearance of LAC. In the aPL+ group, three patients had a stroke or TIA.

In the aPL+ and APS groups 52/81 and 79/84 patients received primary and/or secondary prophylaxis, respectively. In the aPL+ group, one (1.9%) of the patients out of the three who suffered a TIA/stroke belonged to the group of 52 patients receiving primary prevention, and the other two (6.9%) were from the 29 who did not receive prophylaxis. Within the APS group only those did not receive prophylaxis where contraindicated, i.e. gastro-intestinal bleeding, bad compliance, refusal by the patients, or the person's age was over 75 years. In the APS group, two had a myocardial infarction despite anticoagulant therapy with international normalised ratio (INR) around 2.5-3.0, and five had CVA during the 5 years of study, although they too received secondary prophylaxis (4/5 cumarin + aspirin and 1/5 aspirin only). The 7 patients, in this group, with new MI and CVA also had other cardiovascular risk factors: one was a heavy smoker with hypertension, two had type 2 diabetes, hypertension and hyperlipidemia and the remainder had hypertension and hyperlipidemia. Interestingly, during follow-up the 5 APS patients receiving no prophylaxis did not have any thrombotic manifestations. These were women who suffered foetal losses prior to study baseline and had fluctuating or disappearing aPL positivity during follow-up. *It seems that previous thrombosis and anti-coagulant therapy are the major factors influencing the development of clinical thrombotic events in SLE.*

Data shows the antiphospholipid antibodies' patterns with regards to these being constantly high, fluctuating or disappearing during the follow-up period in the aPL+ and APS groups. There was a statistically significant difference in this pattern between the two groups. I found that in the APS group more patients had constantly high antibody levels.

II. Primary Antiphospholipid Syndrome as the Forerunner of Systemic Lupus

Erythematosus

Computerised cumulative medical records of 362 lupus patients (331 female and 31 male), regularly followed our centre, were retrospectively analysed. Of the total 362 patients 223 were antiphospholipid antibody positive patients and 110 fit the Sapporo and Sydney criteria for antiphospholipid syndrome of whom 26 (7.2%) presented with PAPS and later (median, range: 5.5 (1-29) years) progressed to SLE (PAPS+SLE Group). From the remaining 252 patients without APS (regardless of them presenting with aPL or not) 26 disease duration and gender matched patients were randomly chosen to constitute the SLE only group. And similarly from the remaining 84 SLE patients with SAPS another 26 disease duration and gender matched patients were randomly chosen to constitute the SLE+SAPS group.

Patients in SLE only Group was significantly younger at onset of SLE as compared to the other groups. However, the initial symptoms of APS in PAPS+SLE Group started at about the same age as lupus started in SLE only Group. Both SLE and APS started later in SLE+SAPS Group.

The number of patients affected by deep venous thrombosis (DVT), pulmonary embolism (PE), stroke, transient ischemic attack, vascular lesion on MRI, coronary heart disease (CHD), myocardial infarction (MI) and recurrent foetal loss (RFL) in the different groups were examined. The frequency of these complications was significantly higher in PAPS+SLE Group as compared to SLE only Group, with the exception of PE and MI. The

higher prevalence of thrombotic and obstetrical complications in patients having APS has already been known. Obviously, as a result of the classification criteria, primary APS patients who evolve into lupus will have to have more thrombotic complications as a matter of definition. However, the number of patients with and the rate of RFL were significantly higher in PAPS+SLE Group, also when comparing to SLE+SAPS Group, indicating a particular feature for lupus patients starting with PAPS. On comparison to SLE only Group, the prevalence of DVT, PE, CHD and RFL was significantly higher in SLE+SAPS Group. These differences were observed despite of adequate anti-thrombotic and anti-platelet therapy in patients with secondary APS.

The frequency of clinical symptoms and laboratory signs included in the classification criteria of SLE were registered in all cases. No statistical significant differences were found between the three groups (data not shown). The only exception was kidney involvement, especially WHO type III+IV glomerulonephritis, where patients without APS (SLE only Group) had the highest prevalence of proliferative lupus nephritis. In addition, they also had the highest inflammatory activity, requiring hospitalisation, subsequently receiving the highest doses of methylprednisolone and required cyclophosphamide twice as frequently as patients in PAPS+SLE Group. *This observation may lead to the cautious conclusion that the presence of primary APS may modify the disease outcome in SLE reducing the inflammatory potential. This can only be observed when APS precedes lupus. If the APS develops after SLE it does not modify clinical manifestations and inflammatory activity of lupus.* My results suggest that PAPS may be a forerunner of SLE, but we hypothesise that *PAPS may also co-exist with SLE as an independent autoimmune disorder.* This hypothesis may be supported particularly by the relative long latency between APS and SLE in our 26 cases, and by the significant different disease characteristics observed in comparison with the other two groups. Genetic examinations and extended immunologic examinations (e.g. the presence of ribosome

P protein antibodies) (18) may give further insight into the background of the observed between-group difference. Present observations indicate the importance of follow-up the patients with APS by immunologic respect, especially when APS patients have cerebrovascular manifestation.

In the present study, besides anti-cardiolipin and anti-beta2-glycoprotein I, antibodies directed against phosphatidil-serine, prothrombin and annexinV were measured by commercial ELISA kits in 85 randomly selected lupus patients, 14 of whom met the criteria of antiphospholipid syndrome. Corralations were determined between the presence and concentration of rare antiphospholipids and those included in the diagnostic criteria of antiphospholid syndrome, as well as with clinical trombotic manifestations.

III. Detection of rare phospholipid/co-factor antibodies in lupus patients

Besides anti-cardiolipin and anti-beta2-glycoprotein I, antibodies directed against phosphatidil-serine (PS), prothrombin (PT) and annexinV (AnxV) were measured by commercial ELISA kits in 85 randomly selected lupus patients, 14 of whom met the criteria of antiphospholipid syndrome as well.

Anti-cardiolipin IgG was positive in 14 patients, aCL IgM in 8, anti- β 2GPI IgG in 4 and IgM in five patients. Lupus anticoagulant was detected in 9 cases. Seven patients were positive for anti-phosphatidilserine IgG, 9 for aPS IgM, anti-prothrombin IgG was positive in 9 cases. Anti-prothrombin IgM and anti-annexinV were negative in all patients.

I compared the frequency and the comcentration rare antiphospholipid/co-factor antibodies with each other and with those of aCL, LAC, and a β 2GPI antibodies. *The levels of aPS and aPT were significant higher in patients with LAC. Significant correlation was found between aCL IgG- aPS IgG and aCL IgM-aPS IgM antibodies.* The frequency and the concentration of rare anti-phospholipid/co-factor antibodies were higher in patients with

secondary antiphospholipid syndrome. The presence of such rare antiphospholipid antibodies cumulated in patients with antiphospholipid syndrome. Their presence increased the frequency of thrombotic events in the entire study population, furthermore in those positive for lupus anticoagulant or anti-cardiolipin.

Myocardial infarction, stroke/TIA, deep vein thrombosis, pregnancy loss, valvulopathy thrombocytopenia were more frequent with the presence of rare aPL antibodies.

The rare anti-phospholipid/co-factor antibodies were found in 12% of an un-selected cohort of lupus patients. Their presence was more frequent in patients with secondary antiphospholipid syndrome, and further increased the risk of thrombotic complications.

SUMMARY OF NEW FINDINGS

1. The development of thrombotic processes is increased by the followings:
 - a. The presence of lupus antioagulant is stronger risk than the presence of anti-cardiolipin antibody.
 - b. Thrombotic processes occurre more frequently when IgG isotype anti-cardiolipin is present as compared to the presence of IgM anti-CL.
 - c. The presence of different type antiphospholipid antibodies in the same serum sample further increases the risk a thrombotic events.
 - d. Clinical complications associate to constantly high aPL antibody concentration.
2. Aspirin, used as a primary prophylaxis in antiphospholipid pathients without previous thrombotic event may effectively prevent the development of such complications.
3. Antiphospholipid antibodies may be present preceding SLE, and as such primary antiphospholipid syndrome may be a forerunner or the iniciative phase of lupus. However, APS may associate to SLE as an independent systemic autoimmune disorder, modifying the outcome of both disaeases, especially those of SLE.
4. Rare anti-phospholipid/co-factor antibodies (e.g. those against annexin V, phosphatidyl-serine and prothrombin) can be detected in SLE patients. These associate with each-other and with the traditional, critierial aPL antibodies further increasing the prevalence of clinical thrombotic manifestations.

PRACTICAL SIGNIFICANCE OF THE RESULTS

1. Regular measurements and follow-up of antiphospholipid antibodies, including both anti-cardiolipin, anti-β₂-glycoprotein I, and lupus anticoagulant are required in patients with systemic lupus erythematosus.
2. Patients with constantly high antiphospholipid antibody titer are offered to be treated with aspirin for the reason to prevent the development of thrombotic clinical complications.
3. Patients with primary antiphospholipid antibody syndrome require regular medical follow-up to observe when they progress to systemic lupus erythematosus.

Publication

Articles that directly grounded the PhD theses:

1. **T Tarr**, G Múzes, E Pittlik, G Lakos, P Soltész, T Csépany, M Zeher, G Szegedi, E Kiss: Is the primary antiphospholipid syndrome a forerunner of SLE? Orv. Hetilap 146:203-207, 2005. (in Hungarian)

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Articles in extenso published or accepted for publication: 18

In English:	8
In Hungarian:	10
First author:	10
Impact factor:	18,624