

SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

**Changing of disease course and evaluation of non thrombotic cardiac
manifestations of patients with systemic lupus erythematosus**

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1. Introduction

Systemic lupus erythematosus (SLE) is a chronic systemic autoimmune disease potentially affecting various tissues and organ systems such as cutaneous, renal, cardiopulmonary, musculoskeletal, neural, and hematologic systems. The course of the disease usually consists of alternating periods of remission and exacerbation of mild to moderate severity, which can lead to serious long-term consequences.

Antiphospholipid syndrome (APS) is an autoimmune, thrombo-inflammatory disease characterized by the production of circulating antiphospholipid antibodies (APAs), which play central role in the blood clot formation in arteries, veins and small vessels. APAs are a heterogeneous group of immunoglobulins, including lupus anticoagulant (LA), anti-cardiolipin antibodies (aCL), and anti- β 2-glycoprotein-I antibodies (a β 2GPI), directed against phospholipids, cofactors or phospholipid-cofactor complexes. The detection of their presence is important in the diagnosis of the syndrome, nevertheless, the presence of APAs without any relevant clinical symptoms does not establish an APS diagnosis. Deep veins, pulmonary vessels, and arteries are the most common sites of thrombosis in APS. Furthermore, pregnancy morbidities such as miscarriage, intrauterine fetal death, preeclampsia and eclampsia resulting in premature birth are also common clinical manifestations. The disease can occur as primary APS, but when associated with a systemic autoimmune disease, most commonly SLE, the syndrome is defined as secondary APS.

The relationship between the development of APS and SLE is quite close. Based on the Euro Phospholipid Project's data, 36 % of APS patients suffer from SLE as well. According to other reports, 20-40 % of SLE patients carry antiphospholipid antibodies, out of whom 50-70 % will evolve into patients with definitive APS within 20 years. The connection between the two diseases is also shown by the fact that patients diagnosed with primary APS may later develop SLE. Both disease spectrums have several clinical manifestations overlapping such as hemolytic anemia, thrombocytopenia, leuko-lymphopenia, neurological symptoms, renal impairment or livedo reticularis. It was demonstrated that the development of secondary APS in lupus patients worsen chronic organ damages and increases mortality. The incidence of cardiovascular morbidities and thrombosis is increased in SLE patients compared to the general population, and the accelerated atherosclerosis may be explained by lupus associated risk factors in addition to traditional risk factors. The presence of APAs further increases the chance

of vascular diseases. Deep vein thrombosis, pulmonary embolism, pregnancy morbidities, heart valve disorders, pulmonary hypertension, thrombocytopenia, hemolytic anemia, nephropathy and cognitive dysfunction are more prevalent in APA positive lupus patients than in APA negative ones. Therefore, thrombosis prophylaxis is particularly important in SLE patients with associated APS. Nevertheless, the results of studies on primary prophylaxis in APA positive SLE patients are controversial; furthermore, studies on immunomodulant and immunosuppressive treatments in APS patients are limited only to case reports and case series. Our workgroup has previously investigated the relationship between SLE and APS; however, new therapeutic recommendations have been introduced in the recent decades, which could improve the course of disease and quality of life.

It is known that antiphospholipid antibodies affect the development of cardiac manifestations, but the exact pathomechanism is still not fully understood. It is also known that antiphospholipid antibodies contribute not only to the development of thrombotic events, but also to the accelerated atherosclerosis. APS may cause cardiac thrombotic events such as myocardial infarction, but in rare cases intracardial thrombus formation can also occur. Non-thrombotic clinical manifestations can also develop such as valvulopathies, dilated cardiomyopathy or pulmonary arterial hypertension. The association of SLE with APS or antiphospholipid antibody positivity may increase the risk of cardiac manifestations. Several clinical symptoms may develop in both diseases during the disease course. Some of the cardiac manifestations cause clinical symptoms only late, therefore SLE patients should be screened for cardiac damage even in asymptomatic cases.

The Global Anti-phospholipid Syndrome Score (GAPSS) is used to estimate the risk of recurrent thrombosis, which takes into account the traditional risk factors such as hypertension and hyperlipidemia, as well as the presence of antiphospholipid antibodies (LA, aCL IgG and/or IgM, a β 2GPI IgG and/or IgM and anti-phosphatidylserine/prothrombin complex IgG or IgM). In the case of GAPSS above 10, the risk of developing a thrombotic event is high, but there is no data on whether it is predictive of the development of non-thrombotic APS manifestations. In order to shed light on the achievements of the last decades, first aims of our study was to compare the wide spectrum of laboratory and clinical data of SLE patients diagnosed before and since 2004 in order to determine any changes in the disease course.

The objective of our second study was to assess the occurrence of antiphospholipid antibodies, the manifestations of SLE and APS, furthermore the thrombotic and non-thrombotic cardiac morbidities in a large number of our SLE patients.

2. Materials and methods

2.1. First study population

In our retrospective study, we assessed the wide spectrum of clinical and laboratory data of 554 SLE patients, who received regular follow-up care and therapy at the Division of Clinical Immunology, Institute of Internal Medicine, Faculty of Medicine, University of Debrecen. The diagnosis of SLE was established on the American College of Rheumatology (ACR) classification criteria (1997) or SLICC (2012) criteria, according to the date of diagnosis. Patients diagnosed with SLE before 2012 were revised according to the SLICC criteria for SLE; additionally, all SLE patients enrolled in the present study fulfilled the EULAR/ACR 2019 classification criteria for lupus. The diagnosis of APS was based on the Sapporo (1999) or the Sydney criteria (2006); patients diagnosed with APS before 2006 were revised according to the Sydney criteria.

2.2. Second Study population

The Division of Clinical Immunology, Institute of Internal Medicine, Faculty of Medicine, University of Debrecen is one of the largest tertiary referral centers in Hungary for systemic autoimmune diseases. In our retrospective study, we assessed the data of 369 Hungarian SLE patients who were diagnosed and followed up regularly at our center. All patients enrolled in the study fulfilled the 2019 SLE EULAR/ACR Classification Criteria. We diagnosed APS patients based on the 2006 Sydney Classification Criteria, but the patient fulfilled the revised ACR/EULAR APS classification criteria as well.

2.3. Clinical and laboratory evaluation

All patients were routinely followed up throughout the studied period, and their medical records contained detailed information on medical history, treatments as well as clinical symptoms, physical conditions and laboratory and other findings of each visit. The following demographic and clinical data were analyzed: sex, age, age at diagnosis, disease duration, clinical symptoms and organ manifestations of lupus, comorbidities, laboratory results, immunoserological abnormalities and applied treatments during the disease course. The assessment of chronic organ damage was performed using SLICC/ACR Damage Index (SDI) to identify the chronic

organ damage in lupus patients. As we do not routinely check for anti-prothrombin and anti-phosphatidyl-serine antibodies, we used the adjusted aGAPSS score during our studies. Routine diagnostic laboratory tests were performed at the Regional Immunology Laboratory of the Division of Clinical Immunology and at the Department of Laboratory Medicine, Faculty of Medicine, University of Debrecen. The clinical and laboratory data of patients were extracted from medical documentations and records for statistical analyses.

2.4. Statistical analysis

First study: The IBM SPSS ver. 22.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Chi-square test and Fisher's exact test were used to discriminate between patient groups and In statistics, Cramér's V tests was used to measure of association between two nominal variables. Differences were considered statistically significant at $p < 0.05$.

Second study: The statistical analysis was performed by using SPSS Statistics for Windows, Version 28.0 (IBM Corporation, Armonk, NY, USA) and GraphPad Prism version 9.5 for Windows (GraphPad Software, San Diego, California USA). Kolmogorov–Smirnov, and Shapiro-Wilk normality test were used to determine the distribution of data. In cases of normal distribution, we determined mean \pm standard deviation (SD) values and used two-sample *t*-test for statistical evaluation of the experimental data. In cases of non-normal distribution, median values and interquartile ranges were calculated, and Mann-Whitney *U* test was used. Chi-square test and Fisher's exact test were used to discriminate between patient groups. The rank biserial correlation was used to assess the relationship between a dichotomous categorical variable and an ordinal variable. We determined the optimal cut-off value for aGAPSS with ROC curve analysis. We used two-sided statistical tests, where the *p* value represented a significance rate of <0.05 .

3. Results

3.1. First study

Main Analyses

The study population consisted of 554 Hungarian patients with SLE (496 women and 58 men) with a mean age \pm standard deviation (SD) of 52.2 ± 14.46 years. Their mean age \pm SD at the time of SLE diagnosis was 32.5 ± 12.55 years. We classified the lupus patients into three different groups: (1) APS patients, (2) APA positive patients not fulfilling APS clinical criteria and (3) APA negative patients. 113 patients (20.4 %) were diagnosed with secondary APS, 247 patients (44.6 %) belonged to the APA positive APS negative group, while 194 (35.0 %)

patients formed the APA negative group. All patients were white adults; and the demographic characteristics of the patients' groups did not differ significantly.

The prevalence of antiphospholipid antibodies

Among the antiphospholipid antibodies, anti-cardiolipin (IgG and/or IgM) occurred most frequently (58.48 %), followed by anti- β 2-glycoprotein-I (IgG, IgM and/or IgA) (45.85 %), and lupus anticoagulants (19.49 %). Among SLE patients with secondary APS, 23 patients (20.35 %) were positive for one of the aforementioned markers, 44 (38.94 %) had double positivity, and 46 patients (40.71 %) were positive for all the three laboratory markers; therefore nearly 80% of APS patients were double or triple positive. Regarding APA positive group of lupus patients, 37.5 % of them were single positive, 48.39 % of them were double positive, while 14.11 % of them were triple positive.

The prevalence of clinical manifestations

As expected, the prevalence of thrombotic manifestations were significantly higher in patients with secondary APS, compared to APA negative lupus patients. The prevalence of obstetric complications was also significantly higher in the group of SLE patients with secondary APS; however, it was relatively high also in the group of APA negative lupus patients. We observed no significant difference between the three patient groups regarding other, non-thrombotic, inflammatory organ lesions usually common in SLE. Only central nervous system complications were more common in the secondary APS patients, but this difference compared to the other groups was not significant.

Anticoagulant and antiplatelet therapies

Low dose aspirin (43.72 %) or clopidogrel (4.45 %) were used as primary prevention in the APA positive lupus group. In this group, anticoagulants were given for other indications, such as atrial fibrillation. In the APS group, the largest proportion of patients received vitamin K antagonist therapy. Furthermore, 10.61 % of APS patients receiving DOAC therapy were low-risk patients or had bleeding complications.

Differences between patients of APS group diagnosed with SLE before 2004 or since 2004

As a next step, we formed additional patient groups based on the year of diagnosis in order to compare the disease course of SLE patients diagnosed since 2004 and before. For the sake of

comparability, we collected the data of patients diagnosed before 2004 until January, 2004, therefore, their mean follow-up time was 10.9 years. The patients diagnosed since 2004 were followed-up until January, 2020 for an average of 9.9 years.

Acute myocardial infarction did not occur at all in the patients diagnosed since 2004, while it had developed in 10 out of 76 patients diagnosed with lupus before 2004. The prevalence of Raynaud-syndrome, central nervous system manifestations, polyarthritis and pericarditis significantly decreased in patients diagnosed since 2004. On the other hand, the prevalence of deep vein thrombosis and anemia significantly increased as well as the frequency of lupus anticoagulant in these patients.

Differences between patients of APA positive non-APS group diagnosed with SLE before 2004 or since 2004

We observed significantly decreased prevalence of central nervous system symptoms, psychiatric manifestations, discoid lupus and chronic kidney disease in patients diagnosed since 2004. The ratio of patients showing damage by SDI score also decreased significantly. However, on the other hand, hematologic pathologies such as leukopenia, anemia and thrombocytopenia became more common phenomenon in this patient group.

Differences between patients of APA negative group diagnosed with SLE before 2004 or since 2004

Most changes were similar to the ones observed in APA positive group. Central nervous system manifestations, discoid lupus and pericarditis became less common, while the development of hematological manifestations, including leukopenia, anemia and thrombocytopenia became more frequent in the patients of APA negative group diagnosed with lupus since 2004. The ratio of patients showing damage by SDI score also decreased significantly, although the prevalence of mucous ulcers significantly increased.

Comparison of the medication and disease course of SLE patients diagnosed since 2004 and before

The use of immunosuppressant drugs also shows significant changes. The administration of anti-malarial drugs (44.9 % vs 62 %; $p < 0.0001$), mycophenolate mofetil (5.4 % vs 21.5 %; $p < 0.0001$) and rituximab (1.7 % vs 6 %; $p = 0.049$) significantly increased in SLE patients diagnosed since 2004, while there were no significant changes in the remaining immunosuppressive and immunomodulatory drugs.

During the investigated follow-up period, 21 new thrombotic incidents occurred (14 among patients before 2004, while 7 among patients diagnosed since 2004), from which 19 developed in the APS group, and two in the APA positive group. Among the latter, one developed deep vein thrombosis, while the other developed catastrophic antiphospholipid syndrome (CAPS), so they were transferred to the APS group, leaving 247 patients in the APA positive group out of the original 249. We examined the laboratory profile of the patients suffering from repeated thrombotic manifestations and revealed that all but two patients were double or triple positive for the elements of the APS laboratory criteria, namely lupus anticoagulant, anti-cardiolipin (aCL) and anti-beta2 glycoprotein I antibodies. The severe complications such as HELLP syndrome or catastrophic antiphospholipid syndrome appeared in triple positive patients only.

Comparison of the mortality and cause of death

The mortality in the APS group was significantly higher compared to the other two groups. During the investigated follow-up period, 27 (23.7 %) patients died in the APS group from whom 23 (30.3 %) patients were diagnosed before 2004 and 4 (10.8 %) patients after 2004. There was no difference in the overall mortality rate between the APA positive and the APA negative groups (9.7 % vs. 8.8 %); however when comparing the mortality rate based on the date of diagnosis, a strong improvement was observed in the subgroups of patients diagnosed after 2004, compared to the ones diagnosed before 2004 (APA positive: 3.6 % vs. 12.9 %; APA negative: 0.0 % vs. 14.8 %). Regarding the causes of death, infections, cardiovascular events and tumors were the leading causes in all three groups. The most common cause was infection (33.0 %) in the APS group, followed by cardiovascular mortality (29.6 %) and tumors (18.5 %). In the APA positive group, cardiovascular events (33.3 %), tumors (29 %) and infections (25 %), while in the APA negative group, tumors (41.0 %), cardiovascular events (29.0 %) and infections (23.5 %) was the order.

3.2. Second study

Main analyses

The population of our retrospective study consisted of 369 Hungarian patients with SLE (336 women and 33 men; sex ratio = 10.2 to 1). Their age at the time of SLE diagnosis was 32.3 ± 11.6 years; and they were followed up regularly at our Autoimmune Center for 17.3 ± 10.1 years.

Patients were divided into two groups based on APA positivity. Of the 369 patients included in our study, 258 patients (69.9%) were in the APA positive group, while 111 patients (30.1%)

were in the APA negative group. All patients were white adults; and the demographic characteristics of the patients' groups did not differ significantly.

Antiphospholipid antibodies and other laboratory parameters in APA+ and APA- patients

The a β 2GPI + aCL positivity occurred most frequently (50.0%), followed by a β 2GPI + aCL + LA positivity (23.3%). Isolated aCL positivity was found in 15.5%, isolated a β 2GPI in 4.7%, and isolated LA positivity in 2.7% of the cases. The a β 2GPI + LA and aCL + LA combinations were detected in 1.9% of the cases. 29.8% of all patients were diagnosed with APS.

Several other laboratory abnormalities were found to be more frequent in the APA+ group compared to the APA- group. We observed significant difference in the following parameters: thrombocytopenia (43.0% vs. 30.6%), anemia (82.2% vs. 64.9%), anti-dsDNA (95.7% vs. 82.0%), anti-Sm (41.1% vs. 22.5%), anti-RNP (36.0% vs. 20.7%), anti-SS-A (70.2% vs. 59.5%), ANCA (14.0% vs. 1.8%) and cryoglobulinemia (5.4% vs. 0.9%).

Clinical manifestations and steroid treatment in APA+ and APA- patients

Regarding the organ manifestation and laboratory abnormalities of SLE, the occurrence of alopecia (27.9% vs. 14.4%), central nervous system symptoms (28.3% vs. 12.6%), peripheral nervous system symptoms (12.4% vs. 5.4%) and psychiatric disorders (23.3% vs. 12.6%) were significantly more common in the APA positive group. However, the prevalence of subacute cutaneous lupus (SCLE) was significantly lower in the APA positive group compared to the APA negative group (7.0% vs. 20.7%). Furthermore, the SLICC/ACR SDI value [1 (0-2) point vs. 1 (0-1) point] and also the median cumulative steroid dose [19710 (8760-35030) mg/kg vs. 11680 (4380-27740) mg/kg] were significantly higher in the APA+ group compared to the APA- group.

Cardiac manifestations in APA+ and APA- patients

We detected non-thrombotic cardiac manifestations in 171 (43.6 %) patients among all the examined patients. Valvulopathies were the most common cardiac abnormality. In the group of all patients, mitral insufficiency (MI) was diagnosed in 29.5% of patients, while tricuspid insufficiency (TI) was found in 26.83% of patients. The prevalence of ischemic heart disease (IHD) was 7.86%, aortic insufficiency (AI) was 6.23%, and cardiomyopathy was 5.42%. The proportion of patients who had an acute myocardial infarction (AMI) was 3.52%.

All cardiac anomalies occur more often in the APA+ group, but a significant difference was only in the case of tricuspid (31.4% vs. 18.0%) and mitral insufficiency (33.7% vs. 21.6%). All

cardiac manifestations were more common in the APA + group compared to APA - group, but a significant difference was only in the case of tricuspid (31.4% vs. 18.0%) and mitral insufficiency (33.7% vs. 21.6%). Pulmonary hypertension and Libman-Sacks endocarditis occurred only in the APA + group.

Differences between patients with and without cardiac manifestations

As a next step, we compared the patients with cardiac manifestations with the ones without cardiac diseases. We found that anti-CL IgG (81.4 % vs. 16.3 %), anti-CL IgM (78.3 % vs. 14.7 %) as well as a β 2GPI IgG (74.4 % vs. 17.1 %) and a β 2GPI IgM (68.2 % vs. 15.5 %) were significantly more prevalent in the patients who developed some kind of cardiac manifestation. Double and triple APA positivity did not differ between patient groups.

The prevalence of hypertonia (63.2 % vs. 22.2 %) and hyperlipidemia (49.7 % vs. 16.7 %) were significantly higher in patients with cardiac manifestations, and the frequency of stroke was also significantly higher in this group (10.5 % vs. 3.5 %) (Table 5).

Since valvulopathies were the most common cardiac abnormalities, we also compared patients with and without valvulopathy. In the group of patients with valvulopathies, we observed higher prevalence of aCL IgG (80.9 % vs. 23.1 %), aCL IgM (77.4 % vs. 21.7%), a β 2GPI IgG (73 % vs. 23.8 %) and a β 2GPI IgM positivity (66.1 % vs. 22.4 %) positivity. Additionally, we found higher prevalence of hypertension (50.3 % vs. 27.3 %), hyperlipidemia (50.3 % vs. 19%), ischemic heart disease (13.1 % vs. 4.2 %) and cardiomyopathy (9.8% vs. 2.8 %) in this patient group.

Characteristics of the APA+ patients

In the following, we focused on the APA positive patients (n = 258). Among them, lupus anticoagulant (17.83%) was the least frequently detected, while the occurrence of aCL IgM was 44.57%, aCL IgG 48.45%, a β 2GPI IgM 41.47% and a β 2GPI IgG was 45,35%. A positive aGAPSS was detected in 148 patients (57.36%). We did not find a significant difference in the prevalence of cardiac manifestations when comparing the single APA positive group with either the double APA positive or the triple APA positive group. Deep venous thrombosis as well as definitive APS were diagnosed significantly more frequently in triple APA positive patients.

In the further analyzes for the APA+ group, the cardiac manifestations that at least 10 patients had were included in the statistical analyzes suitable for examining the correlation between individual variables. We examined the associations between the isotypes of each antiphospholipid antibody and ischemic heart disease, mitral prolapse, mitral insufficiency,

tricuspid insufficiency, aortic insufficiency and cardiomyopathy. Figure 2 shows the occurrence of specific antibodies in the presence or absence of various heart diseases. Anticardiolipin IgG positivity was the most common, while lupus anticoagulant positivity was the least common in case of all examined cardiac manifestations.

Regarding IHD, the IgM and IgG isotypes of a β 2GPI and aCL were significantly more prevalent in this condition; however, the effect-size is small. There was no significant difference in the case of lupus anticoagulant, while the aGAPSS values shows a negligible but statistically significant correlation with IHD. Regarding AI, the a β 2GPI IgG and aCL IgM/IgG isotypes are significantly more prevalent in this disorder, but the effect-size is small. There was no significant difference in case of a β 2GPI IgM and LA, while the aGAPSS values shows a negligible but statistically significant correlation with AI. In TI and MI patients, a β 2GPI and aCL IgM and IgG isotypes, as well as LA were significantly more common; and the effect-size is medium-large in case of these antibodies (apart from LA, in which it is small). The aGAPSS values shows a medium-large significant correlation with TI and MI. Regarding PM, the a β 2GPI and aCL IgM and IgG isotypes were significantly more common in this disorder; the effect-size for a β 2GPI immunoglobulins is small, while for aCL immunoglobulins is medium-large. There was no significant difference in case of LA. The aGAPSS shows a weak, but significant correlation with MP. As for CM patients, the a β 2GPI and aCL IgM and IgG isotypes, as well as LA were significantly more prevalent; however, the effect-size is small. The aGAPSS values shows a weak, but significant correlation with CM. Out of all antibodies, the largest effect-size was found in aCL IgG in case of all cardiac manifestations.

Based on our results, we tried to find an aGAPSS score in which a non-thrombotic cardiac manifestation is more likely to occur. In the case of cardiomyopathy, the aGAPSS value above 9.5 points, while in the case of the other investigated cardiac deviations, the aGAPSS value above 8.5 points seems to be predictive of the development of the given cardiac manifestation.

4. Discussion

It is well established that the co-occurrence of systemic lupus erythematosus and antiphospholipid syndrome is a common phenomenon. There are numerous overlaps between the two diseases at both the laboratory and clinical levels, and the development of secondary APS increases the number of arterial and venous thromboembolic events in patients with SLE who are already at higher cardiovascular risk. Among our SLE patients, 20.4 % suffered from

secondary APS, 44.6 % was APA positive without fulfilling the criteria of APS, while 35% did not have antiphospholipid antibodies. Of note, these ratios significantly differ from the results of a Columbian cross-section study, in which the ratio of the three groups appeared as the following: APS 9.3 %, APA positive 30.8 %, APA negative 59.8 %. However, the characteristics of SLE may differ between different ethnicities; and our ratios are similar to the observations based on the Spanish multicenter, hospital-based, retrospective, SLE registry (RELESSER-T). Among the 2398 European patients from the RELESSER-T registry, 1026 (42.8 %) were classified into the SLE group, 555 (23.1 %) into the SLE-APS and 817 (34.1 %) into the APA positive SLE group. Therefore, our results are in line with the observations of the largest European registry of patients with SLE.

Beside thrombotic events, obstetric pathology are the main clinical characteristic of APS; however, we observed a high prevalence of obstetric complications in the group of APA-negative lupus patients as well. The high number of unsuccessful pregnancy in lupus may be explained by a number of non-APS related mechanisms, including complex immunopathological processes, underlying genetic factors, or side effects of previous treatments. Several immune mechanisms play an important role in a normal pregnancy, but in SLE, these immune mediated processes may change and the consequential disturbed immune balance can contribute to the pregnancy complications.

In the present study, we did not find any significant difference in the not APS-related symptoms and other organ manifestations of SLE between the three groups, contrary to the RELESSER-T register's data. Ilgen et al. also found that symptoms specific for SLE such as neurological symptoms, pleuritis, arthritis, nucleolar ANA positivity and endocarditis were more common in the SLE-APS and SLE-APA positive groups than in the APA negative SLE population. Our own data do not confirm these findings.

Most of the patients in the SLE-APS group received anticoagulant therapy permanently, which follows the international protocols; moreover, near one fifth of the patients received both anticoagulants and antiplatelet therapy. Despite these measures taken, repeated thrombotic manifestations appeared in 16.8 % of the patients. According to the data of the PROMISSE study, 44 % of LA-positive APS women suffered pregnancy or obstetric complications even while taking the conservative prophylactic treatment. Saraiva et al. examined an APS population and found that 38.4 % of the patients included in their study developed recurrent thrombosis, of whom 40 % were continuously anticoagulated. The question is whether the use of anticoagulants and antiplatelet agents is sufficient in these cases, or whether other immunosuppressive treatment is also justified. Lifelong use of anticoagulants and antiplatelet

agents is currently the recommended treatment for APS; however, several studies are ongoing regarding the use of immunosuppressive drugs in refractory cases. Immunomodulators and immunosuppressants can be considered, e.g. hydroxychloroquine, adalimumab, belimumab, rituximab, certolizumab, eculizumab, but currently mainly just case reports are available on their effectiveness. There is increasing evidence that antimalarial drugs have a positive effect on antiphospholipid antibodies, and also have a beneficial effect on atherosclerotic processes and reduce chronic organ damage.

Antiphospholipid antibodies play a key role in the development of APS, nevertheless, the data in the literature differ regarding the occurrence of individual antiphospholipid antibodies. The majority of the patients developing recurrent thrombotic events or severe APS complications, such as CAPS or HELLP (Hemolysis, Elevated Liver enzymes and Low Platelets) syndrome showed double or triple antiphospholipid antibody positivity, pointing out that multiple antibody positivity increases not only the thrombosis risk, but also the risk of recurrent thrombosis. This observation is consistent with the data of other studies, which reported that multiple positivity displays higher risk of thrombosis, primarily deep vein thrombosis and pulmonary embolism.

Considering the improving diagnostic protocols leading to an earlier diagnosis, the more differentiated novel immunosuppressive therapies as well as the useful international guidelines, the clinical course of SLE and APS potentially changed a lot in the past two decades. In our study, we focused on the assessment of these changes in disease course of lupus, with a special emphasis on secondary APS and APA positivity. Out of the thrombotic events, we observed an increase in the prevalence of deep vein thrombosis in the last decades, which can be caused by the fact that more attention is paid on the possibly underlying APS and the diagnostic procedure of deep vein thrombosis became more accurate. Acute myocardial infarct did not occur in SLE patients diagnosed in the past 15 years, which can be related to the use of low dose aspirin as primary prevention and the significant increase in the use of chloroquine, which display beneficial effects on atherosclerosis. We did not observe any changes regarding the further thrombotic or non-APS-criteria symptoms. Based on our results, since 2004, hematological complications such as leukopenia, anemia and thrombocytopenia increased significantly in the APA positive and APA negative patient groups, but not in the APS group, therefore these are thought to be manifestations of lupus rather than the consequences of APS. Regarding our total SLE population, the prevalence of both central nervous system involvement and chronic organ damages have been significantly decreased in the past decades, albeit the most central nervous system involvements and organ damages developed in the APS group.

New thrombotic events occurred in the APS group in spite of the use of anticoagulants and thrombocyte aggregation inhibitors. Nineteen (16.8 %) of our SLE-APS patients developed new thrombotic manifestations. The number of new thrombotic events among patients diagnosed before 2004 is twice as high as the number of cases developed in patients diagnosed after 2004. This suggests that the course of SLE-APS did indeed improve, despite the fact that the mortality rate was significantly higher in this patient group. The causes of death were consistent with the data of other centers: cardiovascular morbidities, infections and tumors were the most common causes.

Cardiac manifestations are common in both SLE and antiphospholipid syndrome; nevertheless, only pericarditis is included in the classification criteria of SLE. Formerly, thrombotic arterial event (MI) was the only cardiac manifestation in the criteria for APS, but the new ACR/EULAR classification criteria for APS includes cardiac valve thickening and vegetation as well. In addition to these, many cardiac abnormalities can occur in both diseases. Among the non-criteria symptoms, the most common in both diseases is the development of various valvulopathies. With the spread of echocardiography, the diagnosis of valvular disorders associated with SLE and APS has become more accurate and earlier. The frequency of occurrence varies by cohort, but it affects approximately 40% of patients, as we also found (41.5%). The pathomechanism of valvulopathies is not yet fully understood. Based on the histological examination of the autopsy samples, mononuclear cell infiltration, endothelial cell proliferation, platelet activation and mycothrombus formation can be observed. The presence of antiphospholipid antibodies may promote thrombus formation and immune complex deposition, thus contributing to valve damage. Previous studies confirmed that antiphospholipid antibodies may be associated with cardiovascular diseases in the general population as well. The presence of APAs impacts accelerated atherosclerosis and may lead to cardiovascular complications through multiple mechanisms, such as inhibition of the attachment of the plaque stabilizing and antithrombotic plasma protein, annexin-V to the endothelium, as well as the increase of lipid peroxidation, or the identification of the oxidated phospholipids.

The purpose of our present study was to assess the prevalence of the non-thrombotic cardiac manifestations in SLE patients, evaluate the connection between these disorders and the presence of specific antiphospholipid antibodies. We were also interested in whether the aGAPPS score is predictive of the development of non-thrombotic cardiac manifestations.

All cardiac diseases were more common in the APA positive group of our patient population, but a significant difference was found only in the case of mitral and tricuspid insufficiency. The most common cardiac abnormalities were heart valve defects, including mitral and tricuspid

insufficiency. Similar results were previously described by several working groups, while others found no correlation. Multiple studies examined the relationship between the isotype or titer of antiphospholipid antibodies and valvular diseases, however, data in the literature are controversial. Pons and colleagues found that with the presence of lupus anticoagulant or double antiphospholipid antibody positivity, valvular disease has a higher chance of developing. They also found that valvulopathies are more common in arterial hypertension and arterial thrombosis at the diagnosis of APS, and also in livedo reticularis. They found no difference between the primary APS and secondary SLE-associated APS patients. Djokovics and colleagues analyzed a total of 374 patients, out of whom 260 patients were diagnosed with primary APS and 114 patients had SLE-associated secondary APS. Cardiac manifestations showed significantly higher prevalence in SLE-associated secondary APS patients according to their results, and they found valvulopathies to be the most common diseases, which is in accordance with our results. Valvulopathies showed an association with anticardiolipin IgG and acute heart failure, whereas myocardial infarction associated with lupus anticoagulant.

Analyzing our own patient population, we found that stroke was more common in patients who showed any form of cardiac manifestation. Another working group also found the occurrence of stroke to be more frequent, primarily among SLE patients with valvulopathy. Traditional cardiovascular risk factors such as hypertension and hyperlipidemia were more common among patients with cardiac manifestations, which we know contribute significantly to the development of cardiovascular diseases in addition to disease-specific risk factors.

Our results showed that anticardiolipin IgG and IgM, as well as anti- β 2GPI IgG and IgM were more common in patients with valvular diseases. Lupus anticoagulant did not affect the occurrence of cardiac manifestations, including valvulopathies. We did not find correlation between double or triple antiphospholipid antibody positivity and any specific cardiac diseases. Definitive APS was diagnosed significantly more frequently in patients with triple APA positivity, however this did not have an impact on the development of thrombotic and non-thrombotic cardiac events. Based on our data, we found the strongest association between the presence of anticardiolipin IgG antibodies and the non-thrombotic cardiac manifestations. Based on the results of a recently published meta-analysis, patients with anti-cardiolipin IgG antibodies can benefit the most from echocardiography screening tests. At the same time, the incidence of ischemic heart disease and cardiomyopathy was significantly higher in patients with valvulopathy, which draws attention to the fact that non-thrombotic cardiac manifestations can occur not only independently but also in combination with each other.

In our study population, the incidence of ischemic heart disease, aortic insufficiency and cardiomyopathy was less than 10%, while the proportion of patients with acute myocardial infarction was 3.52%. International data show that pulmonary arterial hypertension develops in less than 4% of SLE patients, which is in accordance with our own observations, as PAH occurred in 1.08% of our patient population, noting that this only occurred in antiphospholipid antibody positive patients.

We evaluated the possible correlations between the GAPSS used to estimate the risk of recurrent thrombosis and the development of non-thrombotic cardiac manifestations. Regarding this question, only a few data are available in the literature. Previous studies primarily targeted the association between the aGAPSS and myocardial infarction. A high aGAPSS score may be helpful in assessing the risk of myocardial infarction according to their results. Song et al investigated the aGAPSS score in primary APS patients in relation to the estimation of ischemic stroke. They found that an aGAPSS above 10 significantly increased the risk of stroke. Furthermore, a Spanish working group examined whether the aGAPSS score is predictive for the development of obstetrical complications in patients with antiphospholipid antibody positivity. They found that the aGAPSS score cannot be used for risk estimation in this population, since in addition to antiphospholipid antibodies, several other pregnancy-specific factors influence pregnancy outcome. In our study, we found a positive aGAPSS in 148 patients (57.36%) within the APA+ patient group. IHD, AI showed a negligible, CM a weak, while TI and MI showed a medium correlation with the aGAPSS values. Based on our results, in the case of cardiomyopathy, the aGAPSS value above 9.5 points, while in the case of the other investigated cardiac deviations, the aGAPSS value above 8.5 points seems to be predictive of the development of the given cardiac manifestation. Similar results and studies were not found in the literature.

5. Summary

The relationship between systemic lupus erythematosus and antiphospholipid syndrome is very close. Regarding the clinical manifestations of the two diseases, they might have a very diverse appearance, and many overlaps can be observed between them. The development of secondary APS in lupus patients worsens chronic organ damages and increases mortality. In addition, cardiovascular complications are among the leading causes of death in SLE patients. Cardiovascular complications of SLE might be elevated in the presence of antiphospholipid antibodies (APA) or the association of antiphospholipid syndrome (APS).

In the first part of our work, we compared the laboratory and clinical data of our patients diagnosed with SLE before and after 2004. The examined patients were classified into three groups based on the presence or absence of antiphospholipid antibodies: APA negative, APA positive and the definitive APS group. Among the thrombotic clinical symptoms in the APS group, acute myocardial infarction did not occur among patients confirmed since 2004, while the incidence of deep vein thrombosis increased. Among the clinical symptoms, the frequency of Raynaud's syndrome, central nervous system symptoms, polyarthritits, and pericarditis decreased significantly, while among the laboratory abnormalities, the frequency of lupus anticoagulant and anemia was significantly higher among patients diagnosed after 2004. In the APA positive and APA negative groups, among patients diagnosed after 2004, the frequency of central nervous system symptoms decreased significantly, the frequency of hematological abnormalities increased, but the number of patients with a SLICC impairment score decreased significantly in both groups. Regarding the patients who suffered repeated thrombotic events, we found that, excepting of two patients, the patients were double or triple APA positive. Mortality in the APS patient group was significantly higher compared to the other two groups. In the second part of the work, we focused on cardiac manifestations. All cardiac abnormalities occurred more frequently in the APA+ group, but a significant difference was found only in tricuspid and mitral regurgitation, while pulmonary hypertension and Libman-Sacks endocarditis occurred only in the antiphospholipid antibody positive group. Comparing patients with and without at least one cardiac abnormality, we found that patients with cardiac abnormalities had a significantly higher prevalence of aKL IgG and IgM and a β 2GPI IgG and IgM. Double or triple APA positivity did not influence the occurrence of cardiac manifestations. Hypertension, hyperlipidemia, ischemic heart disease and cardiomyopathy were significantly more often associated with valvulopathy. During the examination of APA positive patients, we found that the aKL IgG antibody shows the closest correlation with any non-thrombotic cardiac manifestations. The ischemic heart disease, the aortic insufficiency showed negligible, the cardiomyopathy weak, while TI and MI showed a significantly moderate correlation with the aGAPSS score. The aGAPSS score above 9.5 points was predictive of cardiomyopathy, while a score above 8.5 was predictive of other cardiac abnormalities.

6. Summary of new observations

1. Acute myocardial infarction did not occur at all in the patients diagnosed since 2004, however the prevalence of deep vein thrombosis significantly increased, we observed significantly decreased prevalence of central nervous system symptoms independently of antiphospholipid antibodies since 2004.
2. The ratio of patients showing damage by SDI score decreased significantly in the group of APA- and APA+, but did not change in APS group
3. The mortality in the APS group was significantly higher compared to the other two groups.
4. All cardiac anomalies occur more often in the APA+ group, but a significant difference was only in the case of tricuspid and mitral insufficiency, the largest effect-size was found in aCL IgG in case of all cardiac manifestations.
5. The prevalence of hypertonia, hyperlipidemia, ischemic heart disease and cardiomyopathy were significantly higher in patients with valvulopathy.
6. IHD, AI showed a negligible, CM a weak, while TI and MI showed a medium correlation with the aGAPSS values. Based on our results, in the case of cardiomyopathy, the aGAPSS value above 9.5 points, while in the case of the other investigated cardiac deviations, the aGAPSS value above 8.5 points seems to be predictive of the development of the given cardiac manifestation.



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Candidate: Nikolett Nagy

Doctoral School: Gyula Petrányi Doctoral School of Allergy and Clinical Immunology

List of publications related to the dissertation

1. **Nagy, N.**, Bói, B., Papp, G., Fiák, E., Gáspár-Kiss, E., Perge, B., Farmasi, N., Tarr, T.:
Antiphospholipid Antibodies Are Major Risk Factors for Non-Thrombotic Cardiac
Complications in Systemic Lupus Erythematosus.
Biomedicines. 12 (3), 1-15, 2024.
DOI: <http://dx.doi.org/10.3390/biomedicines12030530>
IF: 4.7 (2022)
2. **Nagy, N.**, Papp, G., Gáspár-Kiss, E., Diószegi, Á., Tarr, T.: Changes in Clinical Manifestations and
Course of Systemic Lupus Erythematosus and Secondary Antiphospholipid Syndrome over
Three Decades.
Biomedicines. 11 (4), 1-10, 2023.
DOI: <http://dx.doi.org/10.3390/biomedicines11041218>
IF: 4.7 (2022)

List of other publications

3. Szabó, K., Jámbor, I., Pázmándi, K. L., **Nagy, N.**, Papp, G., Tarr, T.: Altered Circulating Follicular T
Helper Cell Subsets and Follicular T Regulatory Cells Are Indicators of a Derailed B Cell
Response in Lupus, Which Could Be Modified by Targeting IL-21R.
Int. J. Mol. Sci. 23 (20), 1-20, 2022.
DOI: <http://dx.doi.org/10.3390/ijms232012209>
IF: 5.6
4. Majai, G., **Nagy, N.**: Egy sokarcú monogénes autoinflammatorikus kórkép, az adenozin-deamináz-
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okokra is.
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6. Tarr, T., Papp, G., **Nagy, N.**, Cserép, E., Zeher, M.: Chronic high-dose glucocorticoid therapy triggers the development of chronic organ damage and worsens disease outcome in systemic lupus erythematosus.
Clin. Rheumatol. 36 (2), 327-333, 2017.
IF: 2.141

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