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Extended Antiphospholipid Antibodies Screening in Systemic Lupus Erythematosus Patients

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Background. The antiphospholipid syndrome (APS) is one of the most encountered autoimmunity in systemic lupus erythematosus (SLE) patients and pathogenesis of these two seems to be intricate.

Aim. To investigate the association of antiphospholipid antibodies (APLAs) titer with the presence of secondary APS diagnosis in SLE patients.

Methods. 65 patients fulfilling the 2012 Systemic Lupus Collaborating International Clinics (SLICC) SLE's criteria were included. The APS diagnosis was sustained according to the 2006 Sydney APS's criteria. Three groups of patients were defined: SLE patients with secondary APS, SLE with history of positive "criteria" APLAs but without APS clinical features, respectively SLE patients without positive APLAs or clinical APS criteria. An extended APLAs panel was searched in all cases: both IgM and IgG of anticardiolipin antibodies (aCL), anti-β2 glycoprotein I antibodies (aβ2GPI), antiphosphatidylethanolamine antibodies (aPE), antiphosphatidylserine antibodies (aPS), respectively antiprothrombin antibodies (aPT).

Results. Only the aβ2GPI, both IgM and IgG serotypes, had significantly higher titers in patients with SLE and secondary APS compared to no APS (with/ without positive APLAs): median (min; max) 7.0 (0.0-300.0) *vs*. 1.0 (0.0-28.0) *vs*. 1.0 (0.0-12.0), respectively 3.0 (0.0-79.0) *vs*. 1.0 (0.0-3.0) *vs*. 1.0 (0.0-12.0) ($p<0.001$, Kruskal-Wallis test)]. Also, in regression logistic models, only the aβ2 GPI (IgG and IgM) were identified as risk factors for secondary APS diagnosis in the SLE patients: OR(95%CI) 5.9 (2.2-15.7), respectively 1.3 (1.1-1.5). In regard with the SLE markers, the IgG serotypes of the "non-criteria" APLAs analyzed (aPS, aPT, aPE) were correlated with the antiDNA titers while the IgM serotypes inversely associated with the complement C3 levels.

Conclusions. IgG aβ2 GPI are accompanied by almost 6-fold increase risk of secondary APS when screening SLE patients. On the contrary, the "non-criteria" APLAs do not seem associated with the APS diagnosis in SLE patients. Some correlates of the "non-criteria" APLAs with the antiDNA and complement C3 levels were also observed.

Key words: Anti-β2 glycoprotein I antibodies, antiphosphatidylethanolamine; antiprothrombine; antiphosphatidylserine; systemic lupus erythematosus; antiphospholipid syndrome.

INTRODUCTION

The "criteria" antiphospholipid antibodies (APLAs) – lupus anticoagulant (LAC), anticardiolipin antibodies (aCL) and anti-β2 glycoprotein I antibodies (aβ2GPI) – are part of both 2012 Systemic Lupus Collaborating International Clinics revised and validated by the American College of Rheumatology (SLICC/ACR) systemic lupus erythematosus (SLE) [1] and 2006 Sydney APS diagnostic criteria [2].

Furthermore, the APLAs are some of the most frequent antibodies encountered in SLE patients, positive in 30 to 40% of the SLE patients. Among the "criteria" APLAs, the aCL are the most common encountered in SLE patients, 47%, followed by the aβ2GPI and LAC, 33% and 26%, respectively [3]. Even so, only one third of these patients will have APS, suggesting that the APLAs positivity is not the only pathogenic link [4]. The APLAs tend to precede the clinical APS events by several years and their positivity characterized a subset of the SLE disease with early disease and severe outcome [5]. Among the APS patients, one third [6] to 45% [7] are secondary to the SLE or have SLE-like disease. These data suggested that the occurrence of both APS and SLE might have common determinants [8] and that the APLAs production might be genetically determined [9].

Apart of diagnostic APLAs, there are also "non-criteria" APLAs, like antiphosphatidylethanolamine antibodies (aPE), antiphosphatidylserine

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antibodies (aPS), antiprothrombin antibodies (aPT) or anti-prothrombin in complex with phosphatidyl-serine antibodies (aPS/PT) that have similar phospholipidic structure with the "criteria" APLAs, but for which the clinical significance remains still uncertain.

In this research, we realized an extended screening for "criteria" and "non-criteria" APLAs in SLE patients, with or without secondary APS, in order to investigate their relevance for the secondary APS diagnostic, as well as the association with the SLE biologic parameters.

METHODS

Subjects' description

Patients fulfilling the 2012 SLICC/ACR SLE's criteria [1] were included. The diagnosis of secondary APS was noted according to the 2006 Sydney APS's criteria [2]. The presence of acute or chronic infections, as well as the overlap syndrome with another connective tissue disease were considered exclusion criteria.

Data regarding the history of any diagnosis criteria of both SLE and APS were collected in all patients. Values of the serum anti-DNA, respectively complement C3 and C4 levels, were noted when present in the patients' files at inclusion.

Groups of patients

After analyzing the antecedents of APS's clinical diagnosis criteria (thrombotic events or pregnancy pathology) as well as those related to the previous APLAs determinations, three groups of patients were defined:

Group 1 – SLE patients with secondary APS (presence of previous thrombotic events or pregnancy pathology and twice or more positive determinations of at least one of the diagnostic APLAs: LAC, aCL or aβ2GPI).

Group 2 – SLE patients without APS's clinical criteria (without previous thrombotic events or pregnancy pathology), but with previous positive APLAs determinations (previous positive results for the LAC, aCL or aβ2GPI; these data were obtained from the patients' files).

Group 3 – SLE patients without APS's clinical criteria (without previous thrombotic events or pregnancy pathology) and without antecedents of positive APLAs (only negative previous determinations of LAC, aCL or aβ2GPI)

APLAs determination

Blood samples were collected at inclusion in all patients; these were centrifuged 15 minutes at 4000 rpm and then stored frozen at -70° in the Immunology Laboratory of Colentina Research Center.

Extended APLAs profile was searched in all cases: IgG and IgM aCL, IgG and IgM aβ2GPI, IgG and IgM aPS, IgG and IgM aPE, respectively IgG and IgM aPT.

All determinations were made by ELISA – Aesku Diagnostics, Wendelsheim, Germany using the analyzer Chemwell 2910, Awareness Technology, Palm City, Florida, USA. For each sample, mean optical density at 450 nm was considered (BioRad Hercules, CA, SUA).

Statistical analysis

The cases characteristics were summarized as mean ± standard deviation for normally distributed variables and as median (minimum; maximum) for those with non-Gaussian distribution. Nonparametric correlation was computed (Kendall's tau coefficient). The differences of the APLAs titers between the three groups defined in our study were analyzed by the Kruskal-Wallis test and the differences between the groups two-by-two were further analyzed, with the utilization of the posttest corrections (Bonferroni). The proportion of the total variance in a dependent variable was assessed by eta-squared (chi-squared obtained by Kruskal-Wallis test divided to "n-1"). Multivariate analysis by logistic regression was realized for determining the APLAs titers as predictors for the secondary APS diagnosis. Each time the logistic regression model included the following variable: gender, age at inclusion, SLE disease duration, anti-DNA and complement C3 levels as well as the titer of the respective APLAs considered into analysis. Odds ratios (ORs) and 95% confidence intervals (CIs) were also calculated. Two-sided p-values less than 0.05 were considered statistically significant. In all analysis, SPSS version 16.0 (Chicago, IL, USA) was used.

RESULTS

Subjects descriptions on the three groups

Data of 28 patients with SLE and secondary APS (Group 1), 8 patients with previous positive APLAs but without antecedents of thrombotic events

or pregnancy pathology (Group 2), respectively 29 SLE patients without history of positive APLAs (Group 3) were taken into analysis (see Table 1).

Both ages at inclusion in the study as well as the SLE disease duration were higher among the patients with SLE and secondary APS than in those only with history of positive APLAs but without clinical APS criteria. The lowest age at inclusion and disease duration were observed in patients with SLE without any biological or clinical features of APS – the patients with SLE and secondary APS had mean (SD) age at inclusion of 45.5 (11.2) and 11.5 years of disease duration, substantially higher than that of the SLE patients with negative APS characteristics, mean (SD) age at inclusion of 41.8 (13.2), respectively 4.0 years disease duration.

Table 1

Subjects description (Group 1 – SLE patients with secondary APS, Group 2 – SLE patients with positive APLAs but without APS's clinical criteria, Group 3 – SLE patients without positive APLAs)

Characteristic	Group 1	Group 2	Group 3
Number	28	8	29
Gender, F/M (%F)	27/1 (96.4)	4/4 (50.0)	27/2 (93.1)
Age at inclusion, years*	45.5 ± 11.2	43.2 ± 14.3	41.8 ± 13.2
SLE's diagnosis age, years*	33.7 ± 9.3	31.4 ± 15.3	36.9 ± 14.2
Disease duration, years**	11.5 (0.3; 32.0)	6.5 (0.1; 20.0)	4.0 (0.5; 18.0)
Anti-DNA titer, UI/mL**	98.7 (6.5–1153.8)	42.8 (9.7–1615.0)	100.0 (9.2–1194.4)
Complement C3, g/L**	1.1 (0.4; 1.8)	1.1 (0.3; 1.3)	0.9 (0.3; 1.5)
Complement C4, g/L**	0.2 (0.0; 0.4)	0.2 (0.0; 0.3)	0.2 (0.0; 0.4)

*mean ± SD
**median (min; max)

Extended APLAs panel in the three groups of patients

We found significantly higher APLAs titers in first group of patients (SLE with secondary APS) when compared with the other SLE patients, both groups 2 or 3, only for two APLAs: aβ2GPI IgM and IgG [median (min; max) 7.0 (0.0-300.0) *vs*. 1.0 (0.0-28.0) vs. 1.0 (0.0-12.0); p<0.001, respectively 3.0 (0.0-79.0) *vs*. 1.0 (0.0-3.0) *vs*. 1.0 (0.0-12.0); p<0.001].

Other four antibodies were found to have significantly higher titers in patients with SLE and secondary APS (group 1) than in patients with SLE and negative previous APLAs (group 3): IgM aCL, IgM aPS, IgG aPS, respectively IgG aPE (see Table 2).

Table 2

The APLAs titers in the three groups of patients (Group 1 – SLE patients with secondary APS, Group 2 – SLE patients with positive APLAs but without APS's clinical criteria, Group 3 – SLE patients without positive APLAs)

Characteristic	Group 1	Group 2	Group 3					
	UI/mL; med(min;max)			p*	η^2**	p(1/2)***	p(2/3)***	p(1/3)***
IgM aCL	4.0(0.0-41.0)	1.0(0.0-29.0)	2.0(0.0-69.0)	**.004**	.174	NS	NS	**<.001**
IgG aCL	1.0(0.0-64.0)	2.0(1.0-28.0)	2.0(0.0-23.0)	.148	.597	NS	NS	NS
IgM aβ2 GP I	7.0(0.0-300.0)	1.0(0.0-28.0)	1.0(0.0-12.0)	**.000**	.307	**.036**	NS	**<.001**
IgG aβ2 GP I	3.0(0.0-79.0)	1.0(0.0-3.0)	1.0(0.0-2.0)	**.000**	.523	**.006**	NS	**<.001**
IgM aPT	4.0(0.0-13.0)	2.0(0.0-26.0)	2.0(0.0-143.0)	.301	.375	NS	NS	NS
IgG aPT	3.5(0.0-20.0)	4.0(2.0-19.0)	4.0(2.0-82.0)	.744	0.09	NS	NS	NS
IgM aPS	4.5(0.0-31.0)	1.0(0.0-28.0)	1.0(0.0-73.0)	**.001**	.222	NS	NS	**<.001**
IgG aPS	2.0(1.0-112.0)	1.0(1.0-14.0)	1.0(1.0-9.0)	**.019**	.124	NS	NS	**.012**
IgM aPE	7.0(0.0-202.0)	2.0(1.0-82.0)	3.0(1.0-26.0)	**.005**	.164	NS	NS	**.006**
IgG aPE	3.0(1.0-151.0)	3.0(1.0-56.0)	2.0(1.0-22.0)	.746	.009	NS	NS	NS

p – significant value < 0.05, NS – non-significant
*Kruskal-Wallis test, **Eta squared; ***with Bonferroni correction applied

Univariate analysis

Statistically significant correlations, but associated with low strength of association as expressed by the Kendall's tau coefficient, were found between the anti-DNA titer and those of theIgG aCL, IgG aPE, respectively IgG aPT antibodies. Moreover, the complement C3 levels were indirectly correlated with the IgM aCL, IgM aPE, IgM aPS, respectively IgM and IgG aPT titers, while the complement C4 levels were significantly negatively correlated only with both IgM and IgG aPT titers (see Table 3).

Multivariate analysis

In regression logistic models adjusted for sex, age, disease duration, anti-DNA titer and complement C3 levels, only the IgG and IgM aβ2 GPI were associated with increased risk of secondary APS in SLE patients. The highest relative risk of secondary APS seems to be associated to the IgG aβ2 GPI, almost 6-fold increase (as presented in Table 4).

Table 3
The correlations of antiDNA antibodies with the APLAs

	IgM **aCL**	IgG **aCL**	IgM **aβ2GPI**	IgG **aβ2GPI**	IgM **aPE**	IgG **aPE**	IgM **aPS**	IgG **aPS**	IgM **aPT**	IgG **aPT**
antiDNA n = 57	NS	p = **.003** r = .271	NS	NS	NS	p = **.048** r = .180	NS	p = **.005** r = .260	NS	p = **.016** r = .221
C3 n = 61	p = **.004** r = -.255	NS	NS	NS	p = **.007** r = -.240	NS	p = **.008** r = -.237	NS	p = **.001** r = -.307	p = **.012** r = -.226
C4 n = 60	NS	NS	NS	NS	NS	NS	NS	NS	p = **.035** r = -.193	p = **.003** r = -.273

*r = Kendall's tau coefficient; p – significant value < 0.05

Table 4
APLA's titers as predictors for the APS's diagnosis by regression logistic by enter method (variables included into regression model: gender, age at inclusion, disease duration, anti-DNA, complement C3 and each time the specific APLA analyzed)

	p-value	**OR (odds ratio)**	**CI (inf – sup)**
IgG aCL	.467	1.032	0.949-1.112
IgM aCL	.060	1.163	0.994-1.361
IgG aβ2 GP I	**.000**	**5.936**	2.240-15.733
IgM aβ2 GP I	**.011**	**1.273**	1.056-1.534
IgG aPE	.689	1.010	0.962-1.061
IgM aPE	.258	1.025	0.982-1.069
IgG aPS	.139	1.150	0.956-1.384
IgM aPS	.118	1.136	0.968-1.332
IgG aPT	.671	0.970	0.843-1.116
IgM aPT	.393	1.065	0.922-1.230

p – significant value < 0.05

DISCUSSION

Both SLE and APS pathologies affect mainly young patients. The mean age at SLE diagnosis as well as that of the SLE population is less than 40 years old [10, 11], 33 years [10], respectively 35 years [11] in some studies. For the APS, the mean age of occurrence is 33 years [6] and the mean age of the study population around 37 years [7]. Moreover, the SLE patients with positive APLAs tend to have early disease onset as well as more severe disease outcome [5]. In our research, the patients with SLE alone when compared to those with SLE and secondary APS had higher SLE's onset age and almost three times longer median SLE's disease duration suggesting the development of the APS in the middle of the SLE immune processes.

Some of the literature data described possible links between APS and SLE. In this regard, it was observed that the prevalence of "non-criteria" APLAs is higher in patients with SLE and secondary APS when compared with SLE without APS's features and their presence seems to increase the risk of thrombotic events [12].

In APS, clinical "non-criteria" APS manifestations, like superficial vein thrombosis, thrombocytopenia, renal microangiopathy, heart valve disease, livedo reticularis, migraine, chorea, seizures or myelitis [13], as well as "non-criteria" APLAs are described. The IgM aPS are more frequently encountered in the SLE patients than in healthy controls and might be correlated with the thrombotic events occurrence [14], or myocardial infarction [15]. The aPS in complex with aPT (aPT/PS complex) are present especially in SLE patients with serum LAC activity [16]. In the aPT and aPT/aPS complex, even different antibodies have partially an identical structure [17]. Analyzing the compounds of the global APS score, the IgG aPS/PT-a component was significantly associated with thrombosis [18]. Also, the aPS/PT might be taken in discussion for inclusion into the "criteria" APLAs [19]. Even if aPE are higher in SLE [14]

and in APS patients [20] than in controls, their titers are not correlated with other APLAs [20]. There are studies that have not found any correlations of the aPE with the clinical APS events [20], but other authors sustained a possible utility in seronegative APS [21].

Searching the pathogenic links between SLE and APS, we analyzed here the correlations of the APLAs titers with the classic biologic markers used to follow-up the SLE patients. For the "non-criteria" APLAs, we found significant correlations for both anti-DNA and complement C3 levels only with the IgG aPT. The anti-DNA correlated also with the IgG serotypes of aPE and aPS. The complement C3, consumed during the immunological processes, was indirectly correlated with the IgM serotypes of aPE, aPS and aPT. Furthermore, among the "non-criteria" APLAs, significant higher titers in SLE with secondary APS when compared with SLE alone are present for aPS and IgM aPE, no significant difference was observed for aPT or IgG aPE.

In regard with the "criteria" APLAs, the LAC was the first described, found to be associated with 6-fold increase of the thrombotic risk [22]. The LAC does not recognize the phospholipids alone [23]; its activity seems to depend on the β2GPI presence [24] and so, it was observed the APS phenotype is not expressed when the β2GPI expression is deficient [25]. The aCL positivity carries a 2-fold increase of venous thrombosis in SLE patients [22]. Apparently, the aCL are not expressed in patients with negative aβ2 GPI [26]. Also, some of the aCL has LAC activity, respectively those β2GPI dependent, but it is still unclear why only some aCL possess LAC activity [27].

Lately, there are data showing that β2GPI is necessary for the antibody with antiphospholipids interaction [28]. For this, the β2GPI interacts with annexins, a family of phospholipid-binding proteins, of which annexin A2 and A5 are involved in APD pathogenesis [29]. Moreover, the pathogenic APLAs seem not to be actually directed against phospholipids, but against the β2GPI [25]. From the β2GPI, the epitope Gly40-Arg43 seem involved in inducing LAC activity and so with the increased thrombosis risk [30].

In this cross-sectional study, even we screened a large number of APLAs, one of the limitations was that we did not have any data on the LAC presence at the moment of the patients inclusion.

In our research, both IgM and IgG aβ2GPI titers were significantly higher in SLE patients that experienced APS's clinical events than in any of the other two SLE groups, suggesting their involvement in the APS's clinical expression. We did not find similar results for the other "criteria" APLAs tested, meaning IgG or IgM aCL. Also, the IgG aβ2GPI associated an almost 6-fold and the IgM aβ2GPI a 1.3-fold increased risk of secondary APS presence in SLE patients.

The presence of the APS pro-coagulant phenotype in patients with positive APLAs is not completely understood and might be related to other factors than APLAs also [29]. The APLAs positivity is not necessarily related to the APS's clinical events, only 8.1% of the patients with positive APLAs will develop a first thrombotic event in 5 years follow-up period [31]. The presence of SLE itself might play a role in thrombosis development as the risk of APS's clinical manifestation is greater in SLE with secondary APS than in primary APS [32]. Other factors than the APLAs positivity were also identified as independent risk factors for APS's clinical events in APS patients, like hypertension [31, 32], hypertriglyceridemia [32], diabetes [33], poverty, higher glucocorticoids doses or damage accrual [34].

CONCLUSIONS

Regarding the possible pathogenic links between the SLE and APS development, we observed that the IgG serotypes of the "non-criteria" APLAs are significantly associated with the antiDNA production while the IgM serotypes with the complement C3 consumption.

The aβ2GPI antibodies seem to be the most reliable APLAs for identification of secondary APS when an extended APLAs screening is applied in SLE patients, the IgG aβ2GPI titer in SLE patients being associated with a 6-fold increased risk of secondary APS.

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Introducere. *Sindromul antifosfolipidic (SAFL) este unul dintre cele mai frecvente fenomene autoimune asociate lupusului eritematos sistemic (LES), iar patogeneza celor două entități pare a fi intricată.*

Obiective. *Investigarea asocierii dintre titrul anticorpilor antifosfolipidici (AAFL) şi prezența SAFL secundar în cadrul LES.*

Material şi metode. *Au fost incluşi în studiu 65 pacienți ce au îndeplinit criteriile SLICC din 2012. Diagnosticul SAFL a fost susținut conform criteriilor Sydney din 2006. Au fost definite trei grupuri: pacienți cu LES şi SAFL secundar, pacienți cu LES şi AAFL pozitivi însă fără manifestări clinice specifice SAFL şi pacienți cu LES fără AAFL pozitivi sau manifestări clinice ale SAFL. Pacienților le-a fost analizat un panel extins de AAFL: anticorpi tip IgM şi IgG anti-cardiolipidici (aCL), anti-β2 glicoproteină I (aβ2GPI), anti fosfatidiletanolamină (aPE), antifosfatidilserină (aPS) şi antiprotrombină (aPT).*

Rezultate. *Numai anticorpii aβ2GPI (atât IgM cât şi IgG) au avut niveluri semnificativ mai mari la pacienții cu SAFL secundar comparativ cu celelalte două grupuri [mediană (min; max) IgM: 7.0 (0.0-300.0) vs. 1.0 (0.0-28.0) vs. 1.0 (0.0-12.0), IgG: 3.0 (0.0-79.0) vs. 1.0 (0.0-3.0) vs. 1.0 (0.0-12.0) ($p<0.001$, test Kruskal-Wallis)]. În cadrul analizei regresiei logistice numai anticorpii aβ2GPI (IgM şi IgG) au fost identificați ca factor de risc pentru diagnosticul SAFL secundar la pacienții cu LES [OR(95%CI) 5.9 (2.2-15.7) pentru IgM, respectiv 1.3 (1.1-1.5) pentru IgG]. Titrurile anticorpilor aPS, aPT şi aPE IgG s-au corelat pozitiv cu cel al anticorpilor anti DNAdc pe când concentrația anticorpilor aPS, aPT şi aPE IgM a fost invers corelată cu nivelurile C3 ale complementului.*

Concluzii. *Anticorpii tip IgG aβ2GPI au crescut riscul de aproximativ 6 ori pentru dezvoltarea SAFL secundar. AAFL ce nu sunt incluşi în criteriile de diagnostic nu par să fie asociați cu diagnosticul SAFL secundar la pacienții cu LES. Aceştia sunt însă corelați cu titrul anticorpilor anti DNAdc şi cu nivelurile serice ale componentei C3 a complementului seric.*

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ABBREVIATIONS

Abs – antibodies
APLAs – antiphospholipid antibodies
APS – antiphospholipid syndrome
aPL – antiphospholipid
aCL – anticardiolipin
aβ2GPI – anti-β2 glycoprotein I
aPE – antiphosphatidylethanolamine
aPS – antiphosphatidylserine
aPS/PT – anti-prothrombin in complex with phosphatidylserine
aPT – antiprothrombin
APS – antiphospholipid syndrome
CRP – C-reactive protein
DVT – deep vein thrombosis
ESR – erythrocyte sedimentation rate
LAC – lupus anticoagulant
SLE – systemic lupus erythematosus

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