ORIGINAL ARTICLE

Added value of non-criteria antiphospholipid antibodies for antiphospholipid syndrome: lessons learned from year-long routine measurements

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Abstract

The international classification criteria for definite antiphospholipid syndrome (APS) include three laboratory measurements: lupus anticoagulant (LA), IgG and IgM isotypes of anti-cardiolipin (aCL) and anti-β2glycoprotein I antibodies (anti-β2GPI). When persistently elevated, they are specific for APS; however, many patients that fulfil clinical criteria may exhibit negative serological results. These "seronegative" APS (SN-APS) are exposed to an increased thrombotic risk. The aims of our cross-sectional, retrospective study of consecutive autoimmune patients' samples were to evaluate the association of non-criteria antiphospholipid antibodies (aPL) with thrombosis and obstetric events, to calculate the risk score for adverse events and to assess the specific contribution of single aPL positivity in SN-APS. LA, aCL, anti-β2GPI and anti-phosphatidylserine/prothrombin antibodies (aPS/PT) of IgG, IgM, and IgA isotypes were determined in sera of 323 patients with autoimmune disorders. Medical records of all patients were carefully analyzed. aCL, anti-β2GPI and aPS/PT of IgG and IgA isotypes were significantly associated with thrombosis while none of the IgM aPL showed such association. aPS/PT of all isotypes, aCL and anti-β2GPI of IgG and IgA isotype showed significant correlation to obstetric events. When considering results of aPS/PT ELISA, we could additionally identify 3% of thrombotic patients and 2% of obstetric patients. Thrombotic and obstetric risk scores were calculated showing significantly higher association to clinical events, as compared to evaluating individual risk factors. aPS/PT could represent an additional biomarker in SN-APS patients. IgA aPL are associated with thrombosis and obstetric complications. Risk scores accounting different aPL and conventional risk factors, better assesses risk for adverse event, as compared to evaluating individual factors alone.

Keywords Antiphospholipid antibodies · Antiphospholipid syndrome · Antiprothrombin antibodies · IgA · Obstetric complications · Thrombosis

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Rheumatology in Slovenia: Clinical practice and translational research

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Introduction

Antiphospholipid syndrome (APS) is an autoimmune disease characterized by recurrent thrombosis or pregnancy complications, along with persistently present antiphospholipid antibodies (aPL) [1]. Lupus anticoagulant (LA), anti-cardiolipin (aCL) and anti-β2-glycoprotein I antibodies (anti-β2GPI), of IgG and/or IgM isotype are the laboratory biomarkers included in the 2006 revised APS classification criteria. However, it is well known that aPL antibodies are heterogeneous in function and specificity, comprising of IgG, IgM and IgA isotypes, and individual tests may recognize various subtypes of antibodies. Furthermore, there are patients strongly suspected of having APS by their clinical phenotype, but persistently

negative for any currently tested aPL [2]. Recently, the concept of "seronegative APS" (SN-APS) has been coined to describe a particular group of patients with clinical manifestation highly suggestive of APS and "non-criteria" aPL, i.e. autoantibodies targeting other plasma proteins or phospholipid-bound proteins complexes [3, 4]. Several authors have suggested that testing for new aPL specificities, may help to identify the syndrome in patients with thrombosis or pregnancy loss.

Antibodies against prothrombin emerged as a potential marker for APS and many reports have confirmed the association between antibodies against phosphatidylserine/prothrombin complexes (aPS/PT) and clinical manifestations of APS. Our group developed an *in-house* aPS/PT ELISA [5] and reported that these antibodies are detected in some of the SN-APS patients and are advantageous in distinguishing patients at greater risk for thrombosis [6–8]. Moreover, we found these antibodies to be the strongest independent risk factor for the aPL-related obstetric complications [8].

The issue of the value of IgA aPL antibodies and their inclusion in the diagnostic algorithm of APS has been a subject of debate since 2006, when the international consensus report concluded that available data for establishing IgA, as an independent risk factor for APS is inadequate. The following literature on IgA aPL is quite diverse, reporting a high variability in its prevalence, as well as clinical significance. However, several experimental data published in recent years showed the potential pathogenic role of IgA aCL and IgA anti-β2GPI [9–14]. These antibodies have been reported in up to 70% of patients with systemic lupus erythematosus (SLE) and in those with primary APS [15].

Recently, two research groups proposed a quantitative index in an attempt to quantify the probability of thrombosis in APS. The aPL score (aPL-S), comprising aPL profile [16] and the global APS score (GAPSS), comprising both aPL profile and conventional prothrombotic risk factors [17]. Both groups included LA, aCL, anti-β2GPI of IgG and IgM isotype as well as IgG/M aPS/PT; however, none included IgA aPL measurements in their calculations. Two validation studies have previously been published in order to support clinical assessment of GAPSS [18, 19].

In order to evaluate the advantage of detection of non-criteria aPL, including IgA aCL, IgA anti-β2GPI and IgG/M/A aPS/PT for patients with chronic rheumatic diseases, their added value for diagnostic and risk purposes was determined. Our aims in this study were first, to systematically record conventional risk factors for thrombotic events, together with aPL positivity and evaluate their association to thrombosis in patients with different systemic autoimmune diseases. Secondly, to analyze the value of non-criteria aPL in the APS risk calculations, separately for thrombosis and obstetric manifestations

and thirdly, to assess the specific contribution of single aPL positivity in SN-APS.

Materials and methods

Participants

This retrospective, cross-sectional study included all patients who were tested for aPL at the Immunology laboratory between January 2014 and November 2014. The laboratory is part of the Department of Rheumatology, University Medical Centre Ljubljana. The study was approved by the National Medical Ethics Committee, Ljubljana, Slovenia (#99/04/15), and all patients provided informed consent.

Among 804 consecutive patient samples tested for aPL, clinical data was obtained for 323 patients (230 females, 93 males) (Table 1). Of these, 106 patients had APS, specifically, 91 primary APS and 15 APS associated to other autoimmune disease. Non-APS patients ($n = 217$) had the following diagnoses: cerebrovascular insult (18), SLE (53), rheumatoid arthritis (14), Sjögren's syndrome (34), systemic sclerosis (7), spondyloarthritis (34), giant cell arteritis (26), other vasculitis (25) and polymyalgia rheumatica (6).

Data collection

Patient medical records were analyzed and the following was recorded at the time of obtaining sera: age, gender, obesity (body mass index > 25 kg/m^2), smoking status, ascertain by self-report (*smokers*, *ex-smokers* and *non-smokers*), arterial hypertension (systolic pressure ≥ 140 mmHg, or diastolic pressure ≥ 95 mmHg, on at least two occasions, or use of oral anti-hypertensive drugs), treatment status including oral contraception (current or at the event), diabetes (fasting glucose value ≥ 7.0 mmol/L on at least two occasions or use of insulin or oral hypoglycaemic medication), history of venous or arterial thrombosis and history of obstetric complications, included in the APS classification criteria [1].

Serological parameters

At the time of the visit, patient sera were measured for aPL profile including LA, aCL, anti-β2GPI, aPS/PT of IgG, IgM and IgA isotype. Lipid levels and platelet count were regularly recorded. Hyperlipidaemia was defined as serum total cholesterol > 5.2 mmol/L or LDL > 3.5 mmol/L, or use of cholesterol-lowering medications and thrombocytopenia was defined as platelet count $< 140 \times 10^9$/L.

Table 1 Patients' demographic and clinical characteristics

No. of patients	Sex (F:M)	Age	Thrombosis	Obstetric complications
APS (91)	65:26	44 (23–85)	70 (77%)	33 (51%)
APS associated to other autoimmune disease (15)	14:1	47 (27–85)	13 (87%)	3 (21%)
Cerebrovascular insult (18)	13:5	46 (21–69)	18 (100%)	0
Systemic lupus erythematosus (53)	45:8	42 (18–77)	2 (4%)	3 (7%)
Rheumatoid arthritis (14)	12:2	54 (24–83)	2 (14%)	0
Sjogren's syndrome (34)	30:4	55 (18–84)	1 (3%)	1 (3%)
Systemic sclerosis (7)	6:1	66 (49–82)	0	0
Spondyloarthritis (34)	17:17	46 (22–75)	0	3 (18%)
Giant cell arteritis (26)	18:8	72 (41–89)	4 (15%)	0
Other vasculitis (25)	12:13	55 (17–86)	4 (16%)	0
Polymyalgia rheumatica (6)	3:3	71 (58–81)	2 (33%)	0

Anti-phospholipid antibody measurements

In-house aPS/PT ELISA In-house aPS/PT ELISA was performed as previously described [20]. Briefly, medium-binding microtitre plates were coated with phosphatidylserine in chloroform/methanol 1:4 and blocked with 1% bovine serum albumin (BSA) in Tris-buffered saline (TBS) containing 5 mM $CaCl_2$ (1% BSA/TBS-Ca). Human prothrombin (Enzyme Research Laboratories, Ltd., Swansea, UK) [10 mg/L] and patients' sera diluted 1:100 in 1% BSA/TBS-Ca were applied to wells immediately one after the other and incubated for 1 h. After washing with 5 mM $CaCl_2$-TBS-0.05% Tween 20, alkaline phosphatase-conjugated goat anti-human IgG/IgM/IgA (ACSC, Westbury, USA) were applied in 1% BSA/TBS-Ca and incubated for 30 min. Binding of antibodies was detected with para-nitrophenylphosphate (Sigma Chemical Company, St. Louis, USA) in diethanolamine buffer (pH 9.8) and OD_{405} was kinetically measured by a spectrometer (Tecan Sunrise Remote, Grödig, Austria).

In-house aCL ELISA IgG, IgM and IgA aCL were determined according to the previously described method [21]. Briefly, medium-binding microtitre plates were coated with cardiolipin and blocked with 10% fetal bovine serum (FBS) in phosphate-buffered saline (PBS). After washing with PBS, diluted samples in 10% FBS-PBS were applied and incubated at room temperature (RT) for 2.5 h. The detection system was the same as in aPS/PT ELISA.

In-house anti-β2GPI ELISA In-house anti-β2GPI ELISA was performed as previously described [22] and evaluated through the European forum for aPL [23]. Briefly, high-binding polystyrene microtitre plates coated with 50 μl/well of β2GPI (10 mg/L) in PBS were incubated for 2 h at RT. The plates were then washed with PBS containing 0.05% Tween-20 (PBS-Tween) and incubated with samples diluted in PBS-Tween for 30 min at RT. The detection system was the same, as in aPS/PT ELISA.

Lupus anticoagulant The assay was performed in blood samples collected in tubes containing 0.109 M sodium citrate. Platelet-poor plasma was obtained by centrifugation at 2400g for 20 min at 4 °C. After filtration, aliquots were stored at − 80 °C until use. Clotting tests were performed using coagulation analyzer BCS Siemens, according to the previous guidelines of the International Society on Thrombosis and Haemostasis ISTH [26]. Simplified Dilute Russell's Viper Venom Test (dRVVT) was performed using LA1 screening reagent and LA2 confirmatory reagent (Siemens) following manufacturer's instructions [27]. A dRVVT ratio (LA1 screen/LA2 confirmation) above 1.2 was considered positive for LA activity. Activity of LA was quantified as follows: low positive (LA1/LA2 = 1.2–1.5), medium (LA1/LA2 = 1.5–2.0) and high positive (LA1/LA2 > 2.0).

Statistical analysis

Categorical variables were presented as numbers and percentages, and continuous variables as means. The significance of baseline differences was determined by the χ^2 test or the unpaired t test, as appropriate. The results of multivariate logistic models were approximated by odds ratio with its 95% confidence interval (OR [95%]). A two-sided $P < 0.05$ was considered statistically significant.

Univariate and multivariate logistic regression analyses were used to determine the contribution of a different variable to a specific condition. To calculate and develop the thrombotic risk score (TRS) and the obstetric risk score (ORS), we followed the previously used model for GAPSS score calculations [17]. Briefly, specific risk factors, identified with univariate analysis, were assigned weighted points calculated as its corresponding β-regression coefficient divided by the value of the lowest β-coefficient (βx/βmin). A risk score was then calculated for each patient by adding together the specific weighted points. The receiver operating characteristic (ROC) analysis and the area under the curve (AUC),

Table 2 Association of prothrombotic risk factors and aPL with thrombosis

	n	Patients without history of thrombosis (207) n (%)	Patients with history of thrombosis (116) n (%)	OR (95% CI)	P	AUC	β-coefficient	TRS weighted points (βx/βmin)	GAPSS weighted points [25]
Sex F:M (ratio)	323	159:48 (3.3)	76:40 (1.9)						
Mean years (range)	323	50 (17–86)	49 (21–89)		ns				
Prothrombotic risk factors									
Smoking	292	54 (29.3)	29 (26.9)		0.648				
Obesity	275	62 (34.4)	32 (33.7)		0.899				
Hyperlipidaemia	285	42 (24.1)	43 (38.7)	2.0 (1.2–3.3)	0.009	0.578	1.714	3	3
Arterial hypertension	323	63 (30.4)	48 (41.4)	1.6 (1.0–2.6)	0.047	0.570	1.663	3	1
Oral contraceptives	230	4 (2.6)	7 (9.3)	3.9 (1.1–13.7)	0.024	0.538	6.649	10	
Diabetes	296	15 (8.1)	7 (6.3)		0.567				
Thrombocytopenia	311	14 (7.1)	16 (14.2)	2.2 (1.0–4.6)	0.042	0.526	2.091	3	
LA	173	37 (33.3)	32 (51.6)	2.1 (1.1–4.0)	0.019	0.586	1.897	3	4
aCL		45 (21.7)	50 (43.1)	2.7 (1.6–4.4)	< 0.001				5
aCL IgG	323	37 (18.4)	43 (37.1)	2.6 (1.6–4.4)	< 0.001	0.543	1.146	2	
aCL IgM	323	14 (6.8)	11 (9.5)		0.380				
aCL IgA	275	7 (3.6)	13 (13.0)	4.0 (1.5–10.4)	0.002	0.539	1.769	3	
anti-β2GPI		39 (18.8)	41 (35.3)	2.3 (1.4–4.0)	0.001				4
anti-β2GPI IgG	323	24 (11.6)	31 (26.71)	2.7 (1.5–5.0)	0.001	0.551	1.281	2	
anti-β2GPI IgM	323	15 (7.2)	10 (8.6)		0.675				
anti-β2GPI IgA	323	13 (6.3)	18 (15.5)	2.7 (1.3–5.8)	0.007	0.525	0.860	1	
aPS/PT		31 (15.0)	28 (24.1)	1.8 (1.0–3.2)	0.041				3
aPS/PT IgG	323	16 (7.7)	21 (18.1)	2.6 (1.3–5.3)	0.005	0.548	1.736	3	
aPS/PT IgM	323	21 (10.1)	19 (16.4)		0.103				
aPS/PT IgA	323	12 (5.8)	15 (12.9)	2.4 (1.1–5.4)	0.026	0.525	0.649	1	

AUC area under curve, *GAPSS* global APS score, *OR* odds ratio, *TRS* thrombotic risk score

Table 3 Diagnostic accuracy of TRS and GAPSS for thrombotic events

	AUC	Sensitivity (%)	Specificity (%)	NPV	PPV	p value
TRS cut-off = 4	0.649	66	64	0.769	0.507	< 0.001
GAPSS cut-off = 4	0.666	53	78	0.676	0.655	< 0.001

diagnostic sensitivity, specificity and positive and negative predictive value (PPV and NPV, respectively) were used to assess the diagnostic performance of the TRS, GAPSS and ORS. Statistical analyses were performed using the SPSS 15.0 program.

Results

Analysis of prothrombotic risk factors and aPL for thrombosis

Univariate logistic regression revealed that among conventional prothrombotic risk factors hyperlipidaemia ($P = 0.009$), arterial hypertension ($P = 0.047$) and use of oral contraceptives ($P = 0.024$) were significantly associated with thrombosis in our cohort (Table 2). Next, we also found thrombocytopenia ($P = 0.042$) and LA ($P = 0.019$), as well as aCL, anti-β2GPI and aPS/PT of IgG ($P \leq 0.005$) and IgA ($P \leq 0.026$) isotypes to be significantly associated with thrombosis. On the other hand, not of IgM subtypes of aCL, anti-ß2GPI and aPS/PT showed association to thrombosis.

A thrombotic risk score calculation (TRS) was constructed following the same model as used in GAPSS [17]. Multivariate logistic analysis was used for the calculation of

the β-regression coefficient. We assigned a number of points proportional to its β-regression coefficient divided by the value of the lowest β-coefficient ($\beta x/\beta min$) to each of the 11 variables showing significant correlation to thrombosis. Each patient had calculated both scores, TRS and GAPSS, by adding together the specific weighted points. Both, TRS and GAPSS values were significantly higher in thrombotic than non-thrombotic patients (mean TRS 6.6 vs. 3.5 and mean GAPSS 6.9 vs. 3.9; $P < 0.001$), showing similar area under the ROC curve (AUC = 0.649 and AUC = 0.666, respectively) (Table 3).

Analysis of aPL for obstetric complications

Among 235 female patients, 147 patients had a relevant obstetric history. For the remaining patients, they were either not pregnant or data was not available. Forty-three patients had a history of obstetric complications, as included in the APS classification criteria. Specifically, 15 experienced three or more unexplained consecutive miscarriages before the 10th week of gestation, 16 experienced unexplained death of a morphologically normal foetus after the 10th week of gestation, and 12 patients gave premature birth to a morphologically normal neonate before the 34th week of gestation, due to eclampsia, preeclampsia or placental insufficiency. The control group comprised of

Table 4 Association of aPL with obstetric complications

	n	Patients without history of obstetric complications (104) n (%)	Patients with history of obstetric complications (43) n (%)	OR (95% CI)	P	AUC	β-coefficient	ORS weighted points ($\beta x/\beta min$)
Mean years (range)	147	49 (18–86)	40 (28–77)					
LA		14 (29.2)	9 (33.3)		0.707			
aCL		21 (20.2)	23 (53.5)	4.6 (2.1–9.8)	< 0.001			
aCL IgG	147	16 (15.4)	22 (51.2)	5.8 (2.6–12.8)	< 0.001	0.684	5.117	11
aCL IgM	147	7 (6.7)	5 (11.6)		0.324			
aCL IgA	134	1 (1.0)	4 (11.1)	12.1 (1.3–112.4)	0.006	0.550	2.018	4
anti-β2GPI		19 (18.3)	16 (37.2)	2.8 (1.3–6.2)	0.014			
anti-β2GPI IgG	147	11 (10.6)	15 (34.9)	4.5 (1.9–11.0)	< 0.001	0.602	1.013	2
anti-β2GPI IgM	147	6 (5.8)	6 (14.0)		0.099			
anti-β2GPI IgA	147	8 (4.8)	7 (16.3)	3.9 (1.2–13.0)	0.021	0.544	1.770	4
aPS/PT		13 (12.5)	15 (34.9)	3.8 (1.6–8.8)	0.002			
aPS/PT IgG	147	6 (5.8)	12 (27.9)	6.3 (2.2–18.3)	< 0.001	0.599	2.496	5
aPS/PT IgM	147	8 (7.7)	9 (20.9)	3.2 (1.1–8.9)	0.022	0.548	0.463	1
aPS/PT IgA	147	6 (5.8)	8 (18.6)	3.7 (1.2–11.5)	0.016	0.563	2.995	6

AUC area under curve, *ORS* obstetric risk score, *OR* odds ratio

Table 5 Diagnostic accuracy of ORS for obstetric complications

	AUC	Sensitivity	Specificity	NPV	PPV	*p* value
ORS cut-off = 5	0.705	57%	83%	0.808	0.605	< 0.001

104 female patients with different autoimmune conditions, negative for obstetric and thrombotic events.

A univariate logistic regression revealed that all aPL, except LA, aCL IgM and anti-β2GPI IgM, were statistically significantly associated with obstetric complications ($P \leq 0.022$) (Table 4).

The obstetric risk score (ORS) was calculated in the same manner, as TRS. ORS values were significantly higher in patients with a history of obstetric complications compared to patients without obstetric complications (mean ORS 10.07 vs. 2.85 $P < 0.001$), with ROC curve (AUC = 0.705 and diagnostic sensitivity 0.565 and specificity 0.832) (Table 5).

Added value of non-criteria aPL for thrombosis and obstetric complications

We investigated the distribution of aPL positivity in the cohort of patients with a history of thrombosis ($n = 116$) and in the cohort of patients with a history of obstetric complications ($n = 43$).

In the group of patients with thrombosis, 13.0% had IgA aCL, 15.5% IgA anti-β2GPI and 24.1% aPS/PT (Table 2). In the group of patients with obstetric complications, 11.1% had IgA aCL, 16.3% IgA anti-β2GPI and 34.9% aPS/PT (Table 4).

None of the patients was single positive for IgA aCL or IgA anti-β2GPI; however, three patients in the thrombotic cohort and one patient in the obstetric cohort expressed a single aPS/PT positivity (Fig. 1). In our cohort of autoimmune patients, we could additionally identify (3/116) 3% thrombotic patients and (1/43) 2% obstetric patients, when considering results of aPS/PT ELISA.

Discussion

In current clinical practice, aCL and anti-β2GPI detected by ELISA and the LA detected by clotting assays are the laboratory classification biomarkers for APS [1]. There have been many attempts to improve and standardize these methods since each individual method has several drawbacks. In addition, LA measurement is precluded by concurrent anticoagulant treatment [24].

Next, these tests may give negative results, even though a diagnosis of APS is very likely due to typical, often multiple clinical manifestations (SN-APS patients). Clinicians occasionally test for non-criteria aPL such as IgA aCL, IgA anti-β2GPI or IgG/M/A aPS/PT antibodies. Several studies have found an association between IgA anti-β2GPI and an increased risk of thromboembolic vascular disease [9, 12, 25]. A systematic literature review concluded that routine measurement of aPS/PT antibodies of IgG and/or IgM isotype might be useful in supporting data for establishing thrombotic risk of patients with previous thrombosis or SLE [26]. In addition, a very recent international multicentre study advised measurement of IgG aPS/PT that might contribute to a better and more complete identification of patients with APS [27]. Our group recently showed IgA aPS/PT to highly correlate with LA activity and thrombosis [28].

The present study is, to our knowledge, the first to date, analysing IgA aCL and IgA anti-β2GPI as well as aPS/PT of all three isotypes, measured in a routine clinical laboratory practice, evaluating their potential as an added value (e.g. non-criteria aPL). We found a significant association between the presence of IgA aCL, IgA anti-β2GPI and IgG/IgA aPS/PT and thrombotic event. Conversely an association between IgM aPL and thrombosis was not confirmed. Although none of the patients was single positive for IgA aPL, we clearly showed that these antibodies were present in APS patients and were associated with an increased risk for thrombotic vascular events.

Furthermore, three patients (one with a history of arterial thrombosis, the second with a history of venous thrombosis
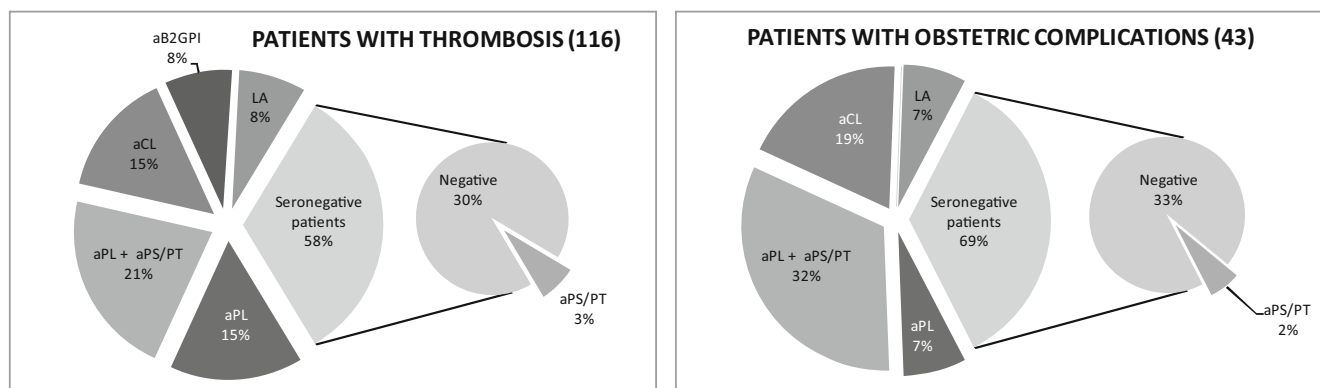

Fig. 1 Distribution of aPL positivity among patients with a history of thrombosis ($n = 116$) and obstetric complications ($n = 43$)

and the third with a history of venous thrombosis and foetal death during the second trimester of pregnancy) expressed single aPS/PT positivity. Besides, aPS/PT levels were persistently elevated in 2 years of follow-up and none of the patients later became positive either for aCL, anti-β2GPI or LA. Therefore, in our cohort of autoimmune patients, we could additionally identify 3% thrombotic patients and 2% obstetric patients, when considering the results of the aPS/PT ELISA. These results confirmed our previous study, where we reported that aPS/PT are associated with adverse pregnancy outcome and that these antibodies are the only aPL associated with early pregnancy complications [8].

Recently, two research groups proposed different quantitative scoring (aPL-S and GAPSS) in which testing for multiple aPL may better evaluate thrombotic risk in APS patients instead of individual aPL measurements [16, 17]. Both risk scores include aPL profiles of IgG and IgM isotypes; however, none comprises IgA measurements. Since IgM isotype of aPL was not independently associated with thromboses in our cohort, but IgA did, we constructed a version of thrombotic risk scoring TRS, that included also the IgA isotype. We found 11 variables to be independently associated with thrombosis including hyperlipidaemia, arterial hypertension, use of oral contraceptives, thrombocytopenia, LA, aCL, anti-β2GPI and aPS/PT of IgG and IgA isotypes. Additionally to TRS, we calculated GAPSS in our cohort of patients for the exact comparison. Both, TRS and GAPSS values were significantly higher in thrombotic than non-thrombotic patients, showing similar area under ROC curve (AUC = 0.689 and AUC = 0.690, respectively). Although different aPL profiles were included in both scoring calculations, no apparent difference was found. In our cohort, GAPSS > 4 produced the highest AUC, which is lower than the cut-off (GAPSS > 10) proposed by the original study [17].

Moreover, in the present study, we are proposing quantitative scoring also for evaluating the risk of adverse pregnancy events in aPL-positive patients namely the obstetric risk score—ORS. Similar to GAPSS and TRS calculations, ORS comprises all with obstetric manifestations significantly associated aPL, specifically aCL and anti-β2GPI of IgG and IgA isotypes, as well as all three isotypes of aPS/PT antibodies. ORS was significantly higher in patients with a history of obstetric events as compared to patients without and showed much higher diagnostic accuracy as compared to each individual aPL measurement. Our results show that scoring calculations can be a suitable quantitative marker for APS; however, we are also suggesting that improved scoring systems are needed.

Conclusions

All non-criteria aPL, including IgA aCL, IgA anti-β2GPI and IgA/IgG aPS/PT were significantly associated to thrombosis.

aPS/PT could represent an additional biomarker in SN-APS patients. Importantly, following 2 years of systematic testing, three patients were serologically identified as single aPS/PT positive. Risk scores including non-criteria aPL in addition to conventional risk factors, better assesses risk for adverse events, as compared to evaluating individual factors alone. An update of the current classification criteria for APS should be considered, incorporating new serological markers into a potential quantitative index, acting as a predictive marker for adverse events.

Take home messages:

- aCL, anti-β2GPI and aPS/PT of IgG and IgA isotype are associated with thrombosis.
- aPS/PT (all isotypes), aCL (IgG and IgA) and anti-β2GPI (IgG and IgA) are associated with obstetric complications.
- aPS/PT could represent an additional biomarker for SN-APS patients.
- Thrombotic and obstetric risk scores better assess risk for adverse events as compared to evaluating individual factors alone.

Compliance with ethical standards

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