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PAPER

Profiling of non-criteria antiphospholipid antibodies in patients with SLE: differentiation of thrombotic SLE patients and risk of recurrence of thrombosis

O Tkachenko[1], S Lapin[1], A Mazing[1], V Emanuel[1], E Belolipetskaia[2], I Beliaeva[2], V Myachikova[3], A Maslyansky[3], P Schierack[4,5] and D Roggenbuck[4,5]

[1]Center for Molecular Medicine, First Pavlov State Medical University of Saint Petersburg, Saint Petersburg, Russian Federation; [2]North-Western State Medical University named after II Mechnikov, Saint Petersburg, Russian Federation; [3]Rheumatology Department, VA Almazov North-West Federal Medical Research Center, Saint Petersburg, Russian Federation; [4]Faculty Environment and Natural Sciences, Brandenburg University of Technology Cottbus-Senftenberg, Senftenberg, Germany; and [5]Faculty of Health Sciences, Joint Faculty of the Brandenburg University of Technology Cottbus – Senftenberg, the Brandenburg Medical School Theodor Fontane and the University of Potsdam, Germany

To reveal the clinical significance of criteria and non-criteria antiphospholipid antibodies detected by line immunoassay in comparison with ELISA, systemic lupus erythematosus patients with and without thrombotic events were investigated. Thus, 107 systemic lupus erythematosus patients (48% with deep vein thrombosis or/and arterial thrombosis) and 120 healthy donors were enrolled. Serum antiphospholipid antibodies were detected by ELISA (Orgentec Diagnostika, Germany) and line immunoassay (GA Generic Assays, Germany). Lupus anticoagulant and IgG to cardiolipin and β2GPI but not IgM as well as triple positivity by ELISA and line immunoassay were linked with thrombosis in systemic lupus erythematosus. IgG to phosphatidylinositol and phosphatidylserine by line immunoassay showed significantly higher levels in systemic lupus erythematosus with deep vein thrombosis/arterial thrombosis than without and were independent risk factors for deep vein thrombosis (odds ratio 3.9, 95% confidence interval 1.1, 13.2) and arterial thrombosis (odds ratio 5.1, 95% confidence interval 1.3, 19.8) as well as thrombosis (odds ratio 3.6, 95% confidence interval 1.1, 11.3) and recurrence thereof (odds ratio 6.9, 95% confidence interval 2.1, 22.6), respectively. The occurrence of >4 IgG antiphospholipid antibodies by line immunoassay was an independent risk factor for thrombosis (odds ratio 10.9, 95% confidence interval 1.2, 101.5), arterial thrombosis (odds ratio 14.6, 95% confidence interval 2.5, 86.3), deep vein thrombosis (odds ratio 5.8, 95% confidence interval 1.0, 32.4) and recurrence of thrombosis (odds ratio 35.9, 95% confidence interval 3.8, 342.8). Line immunoassay is a promising multiplex test for the simultaneous detection of criteria and non-criteria antiphospholipid antibodies. Profiling of antiphospholipid antibodies by line immunoassay can differentiate systemic lupus erythematosus patients with thrombosis from systemic lupus erythematosus patients without and assess the risk for thrombosis and recurrence thereof. *Lupus* (2020) **0,** 1–9.

Key words: Line immunoassay; ELISA; antiphospholipid antibodies; systemic lupus erythematosus; domain 1; deep vein thrombosis; arterial thrombosis

Introduction

Antiphospholipid antibodies (aPLs) are a family of antibodies that interact with PLs, PL-protein complexes and PL-binding proteins. According to the international classification criteria of antiphospholipid syndrome (APS), aPLs can be detected by solid-phase immunoassays and/or by functional coagulation assay (lupus anticoagulant (LA)).[1] In addition to the latter, criteria aPLs encompass IgG and IgM to cardiolipin (aCL) and beta2-glycoprotein 1 (aß2GP1). So-called non-criteria aPL may recognize phosphatidylcholine (aPch), phosphatidylethanolamine (aPe), phosphatidylglycerol

Correspondence to: Olga Tkachenko, Center for Molecular Medicine, First Pavlov State Medical University of St. Petersburg, L'va Tolstogo str. 6–8, Saint Petersburg, 197022, Russia.
Email: tkachenie@mail.ru
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(aPg), phosphatidylinositol (aPi), phosphatidylserine (aPs), annexin V (aAnV) and prothrombin (aPt) as respective targets with or without the mediation of cofactors such as ß2GP1.[2,3]

Apart from being criteria aPLs of APS, aCL, aß2GP1 and LA also represent laboratory criteria of systemic lupus erythematosus (SLE). However, aPLs can be detected in patients with infections and other systemic autoimmune disease as well as elderly patients without any clinical manifestation of APS.[4–7]

In general, aPLs such as aCL and aß2GP1 are analysed by classical ELISAs.[1] However, a disadvantage of aPL testing by ELISA appears to be the 'oversensitivity' of such assays detecting both pathogenic and non-pathogenic, mostly 'low-titre' aPLs not associated with clinical features of APS.[8,9] Thus, to exclude transiently non-pathogenic aPLs, positive findings should be confirmed after 12 weeks by medium or high titres ($\geq$40 U or $\geq$99th percentile).[1]

An emerging method for aPL analysis is the line immunoassay (LIA).[10] This test employing a hydrophobic polyvinylidene fluoride (PVDF) membrane as the solid phase enables multiplex aPL testing.[10,11] This aPL profiling offers the additional assessment of non-criteria aPLs such as aPch, aPe, aPg, aPi, aPs, aAnV and aPt for the serological diagnosis of patients with APS. The novel assay technique preferably detects aPLs to domain 1 (aD1) of ß$_2$-glycoprotein 1 and, thus, appears to differentiate patients with APS from those with infections and asymptomatic carriers.[12]

The presence of aPL in SLE patients with and without thrombosis is poorly understood.[13] Non-criteria aPLs such as aPg appear to be able to discriminate APS from systemic autoimmune rheumatic diseases by interacting with ß2GP1 exposing D1 epitopes thereof.[14] Hence, the aim of our study was to reveal the clinical significance of aPL profiles detected by LIA in comparison with ELISA in SLE patients with and without thrombotic events.

Patients and methods

Patients and controls

We collected clinical data along with serum and plasma samples from 107 patients with SLE and 120 healthy donors (HD) (Table 1). The diagnosis of SLE was based on standard clinical, laboratory, radiological and histopathological methods in accordance with the Systemic Lupus Erythematosus International Collaborating Clinics classification of 2012.[15] In this cohort, 43% of patients had deep vein thrombosis (DVT) or/and

Table 1 Clinical characteristics of 107 patients suffering from SLE with DVT/AT and those without.

Characteristics	*SLE* (n = *107*)	*SLE With DVT/AT* (n = *47*)	*SLE Without DVT/AT* (n = *60*)	P *DVT/AT vs without DVT/AT*
Age, median years (25–75th percentile)	41 (32–52)	45 (36–54)	37 (32–53.7)	ns
Women, *n* (%)	97 (90%)	43 (91.4%)	54 (90%)	ns
Duration, median years (25–75th percentile)	6 (2–16)	8 (2.5–17.5)	5 (2–15)	ns
Livedo reticularis, *n* (%)	18 (16.8%)	6 (12.7%)	12 (20%)	ns
Malar rash, *n* (%)	43 (40.1%)	18 (38%)	25 (42%)	ns
Arthritis, *n* (%)	72 (67.2%)	31 (65%)	41 (68%)	ns
Pleuritis, *n* (%)	11 (10.2%)	7 (14.8%)	4 (6%)	ns
Renal lesions, *n* (%)	52 (48.5%)	22 (46%)	30 (50%)	ns
Thrombocytopenia, *n* (%)	26 (24.2%)	12 (25%)	14 (23.3%)	ns
Obstetric morbidity, *n* (%)	16 (14.9%)	15 (32%)	1 (1.6%)	<0.0001
AT, *n* (%)	9 (8.4%)	9 (19.1%)	0	<0.005
Venous thrombosis, *n* (%)	28 (26%)	28 (59%)	0	<0.005
Arterial and venous thrombosis, *n* (%)	10 (9.3%)	10 (21%)	0	<0.005
Recurrent thrombosis, *n* (%)	27 (25.2%)	27 (57.4%)	0	<0.005
Obesity, *n* (%)	21 (19.6%)	11 (23%)	10 (16%)	ns
ANA, *n* (%)	79 (73.8%)	32 (68%)	47 (75.8%)	ns
anti-dsDNA antibody, *n* (%)	45 (42%)	17 (36%)	28 (46%)	ns
C4, median g/L (25–75th percentile)	0.2 (0.1–0.3)	0.2 (0.1–0.3)	0.2 (0.1–0.3)	ns
SLEDAI, median (25–75th percentile)	6 (4–12)	8 (4–14)	6 (4–12)	ns

ANA: antinuclear antibodies; AT: arterial thrombosis; dsDNA: double-stranded DNA; DVT: deep vein thrombosis; SLE: systemic lupus erythematosus; SLEDAI: systemic lupus erythematosus disease activity index; ns: non-significant.

arterial thrombosis (AT). Depending on the presence of thrombotic events, we classified SLE patients into two subgroups. The first subgroup included patients diagnosed with SLE and DVT/AT ($n = 47$) and the second group SLE patients without any thrombotic manifestation (SLE without DVT/AT, $n = 60$). In the SLE group with DVT/AT, nine (8.4%) patients had AT, 28 (26%) DVT and 10 (9,3%) both AT and DVT. Further, 16 female patients of this group (14.9%) had pregnancy pathology including early miscarriages, late miscarriages and premature birth. Demographic, clinical and laboratory data are presented in Table 1.

ELISA for the detection of aCL and aß2GPI

For the detection of aCL and aß2GPI in patient sera, commercially available solid-phase ELISAs employing purified human ß2GPI in complex with CL and human ß2GPI were used (Orgentec Diagnostika, Germany). Manufacturer cut-offs were validated as recommended by ISTH.[16] Samples were considered positive when their concentration exceeded the cut-off of 10 U/mL for a CL IgG/IgM and 8 U/ml for aß2GP1 IgG/IgM.

LIA for the detection of aPLs

Antibodies such as aCL, aß2GP1, aPch, aPe, aPg, aPi, aPs, aAnV and aPt were detected in patient serum simultaneously using a commercially available LIA in accordance with the recommendations of the manufacturer (GA Generic Assays, Germany).[12] Processed strips were analysed densitometrically, employing a scanner with the evaluation software DotLine Analyzer (GA Generic Assays). Optical density values equalling 30 or above were scored positive. This cut-off was determined by calculating the 99% percentile of 120 healthy individuals.

LA testing

Analysis of LA was performed in accordance with the international recommendations.[17] Double centrifugation of each sample was performed (first centrifugation at 2000 g, 15 min, at room temperature, then re-centrifuging the plasma for 10 min at a higher speed, >2500 g). For analysis of LA, the following tests were used: activated partial thromboplastin time (APTT), APTT mixing test, Diluted Russell Viper Venom time screen and confirmation tests. All samples that were positive on the screening test were subjected to mixing and confirmatory tests. The results were obtained as the ratio of the screening/confirmatory test (normal range 0.8–1.2). All coagulation tests were performed using Stago tests (France) on the Sysmex CS-5100 System (Siemens Healthcare GmbH, Germany).

Statistical analysis

Data were described as numbers and percentages for categorical variables, and medians with interquartile ranges for continuous variables non-normally distributed. Mann-Whitney *U* or Kruskal-Wallis tests were used for comparison of variables with a non-normal distribution. Fisher's exact test with two-tailed probability was used to test the differences between groups. Inter-rater agreement statistics were applied for comparison of classifications. Rank correlation of variables was performed by Spearman's correlation analysis. Also a logistic regression analysis was performed between one dichotomous dependent variable (thrombosis, AT, DVT, recurrence of thrombotic events) and one or more independent variables including aCl, aß2GPI, aPc, aPe, aPg, aPi, aPs, aAnV, aPt IgG/IgM as well as LA, gender, age, obesity, SLE disease activity index (SLEDAI) and disease duration. GraphPad 8.3.0 statistical software was used for all statistical calculations. A p value <0.05 was considered significant.

Results

Analysis of criteria aPLs

To detect aPL profiles and analyse possible differences in aPL assessment, we tested sera from 107 SLE patients and 120 healthy controls by ELISA and LIA. Comparison of qualitative LIA and ELISA results of criteria aPLs, employing the cut-offs recommended by the manufacturers, revealed good agreement for IgG and IgM to ß2GPI (Cohen's kappa = 0.68 and 0.73 for a ß2GPI, respectively) as well as moderate and fair agreements for aCL IgG and IgM (Cohen's kappa = 0.54 and 0.36, respectively).

The quantitative analysis of aCL and aß2GPI IgG as well as IgM by LIA and ELISA revealed significant correlations with Spearman correlation coefficients (ρ) ranging from 0.32 (aCL IgM), 0.56 (aCL IgG), 0.80 (aß2GPI IgM) to 0.83 (aß2GPI IgG) ($p < 0.0001$).

The group of 47 SLE patients with DVT/AT showed a significantly higher prevalence of aCL IgG detected by ELISA and LIA ($p < 0.005$ and $p < 0.05$, respectively), aß2GPI IgG ($p < 0.005$ for both) compared with the 60 SLE patients without

thrombosis (Table 2). Regarding aCL and aβ2GPI IgM as well as LA, there were no significant differences between SLE with DVT/AT and without. Moreover, no significant differences for all criteria aPLs could be found by comparing SLE patients with AT to those with DVT ($p > 0.05$, data not shown).

Quantitative analysis of classical aPL by ELISA revealed significantly higher aPL levels in SLE with and without thrombosis compared with those in HD ($p < 0.0001$; Figure 1). In contrast, aCL IgG and aCL IgM quantification by LIA did not demonstrate significant differences for the comparison of SLE without thrombosis and HD ($p > 0.05$; Figure 2(a), Figure 2(c)). Concerning aβ2GPI IgM and IgG, quantitative analysis by LIA revealed significantly higher levels in SLE with and without thrombosis compared with those in HD ($p < 0.0001$, respectively; Figure 2(b), Figure 2(d)).

Analysis of non-criteria aPLs

We compared the frequency of 'non-criteria' aPL determined by LIA in SLE patients with DVT/AT and without. In SLE patients with DVT/AT, aPi IgG and aPs IgG were significantly more prevalent than in SLE patients without thrombosis (aPi IgG 23% vs 5%, $p < 0.05$; aPs IgG 25% vs 5.5%, $p < 0.05$). In SLE patients without thrombosis only aAnV IgM was significantly more frequently detected than in patients with (12.7% vs 2.2%, $p < 0.05$). Notably, aPc and aPe were not detected in both groups.

Regarding the differentiation of SLE patients, only aPi IgG detected quantitatively by LIA discriminated SLE with thrombosis from SLE without ($p < 0.05$; Figure 3(c)). The levels of aPs IgG and aPs IgM by LIA also discriminated SLE with thrombosis but not SLE without it from HD ($p < 0.001$, Figure 3(b) and (d)). Quantification of aPa IgM revealed significantly higher aPL levels in SLE with and without thrombosis compared with those in HD ($p < 0.0001$ and $p < 0.05$, respectively).

Analysis of aPL profiles by LIA

Triple and double positives of criteria aPLs in 107 patients with SLE were determined by ELISA/LA or by LIA/LA analyses. We found triple-positive aPL profiles in 25% of SLE patients by ELISA/LA and 19% by LIA/LA, which was not significantly different ($p > 0.05$). There was also no significant difference regarding the analysis of double positives in SLE patients (9% by ELISA/LA vs 14% by LIA/LA, $p > 0.05$).

Triple positives of criteria aPLs analysed by both ELISA/LA and LIA/LA were more frequent in SLE patients with DVT/AT than in patients without (46% vs 9% by ELISA/LA, $p < 0.0001$; 31% vs 9% by LIA/LA, $p = 0.0092$). In this context, double positives of criteria aPLs were only more frequent in case of LIA/LA (18% vs 7% by LIA/LA, $p < 0.05$; 6 % vs 10% by ELISA/LA, $p > 0.05$).

We also evaluated the occurrence of multiple aPL by LIA. In patients with SLE demonstrating DVT/AT, the presence of more than four IgG aPLs was more prevalent in contrast to patients without (25% vs 4%, $p < 0.001$).

Thus, we performed a logistic regression analysis between one dichotomous dependent variable

Table 2 Frequency of criteria antiphospholipid antibodies measured by ELISA, LIA and LA analysis in 107 patients with SLE and 120 HD.

					P	P	P
	SLE (n=*107*)	*SLE with DVT/AT* (n=*47*)	*SLE without DVT/AT* (n=*60*)	*HD* (n=*120*)	*SLE with DVT/AT vs without DVT/AT*	*SLE with DVT/AT vs HD*	*SLE without DVT/AT vs HD*
ELISA							
aß2GP1 IgG	30	20	10	0	<0.0001	<0.0001	<0.0001
aCL IgG	25	18	7	0	<0.0001	<0.0001	<0.0001
aß2GP1 IgM	24	12	12	2	ns	<0.0001	<0.0001
aCL IgM	26	12	14	3	ns	<0.0001	<0.0001
LIA							
aß2GP1 IgG	27	20	7	0	<0.0001	<0.0001	<0.001
aCL IgG	14	10	4	1	<0.05	<0.0001	<0.05
aß2GP1 IgM	22	13	9	1	ns	<0.0001	<0.0001
aCL IgM	10	7	3	0	ns	<0.001	<0.05
LA	42	24	18	5	ns	<0.0001	<0.0001

aCL: antibodies to cardiolipin; aß2GP1: antibodies to beta2 glycoprotein 1; AT: arterial thrombosis; DVT: deep vein thrombosis; HD: healthy donors; LIA: line immunoassay; LA: lupus anticoagulant; SLE: systemic lupus erythematosus.

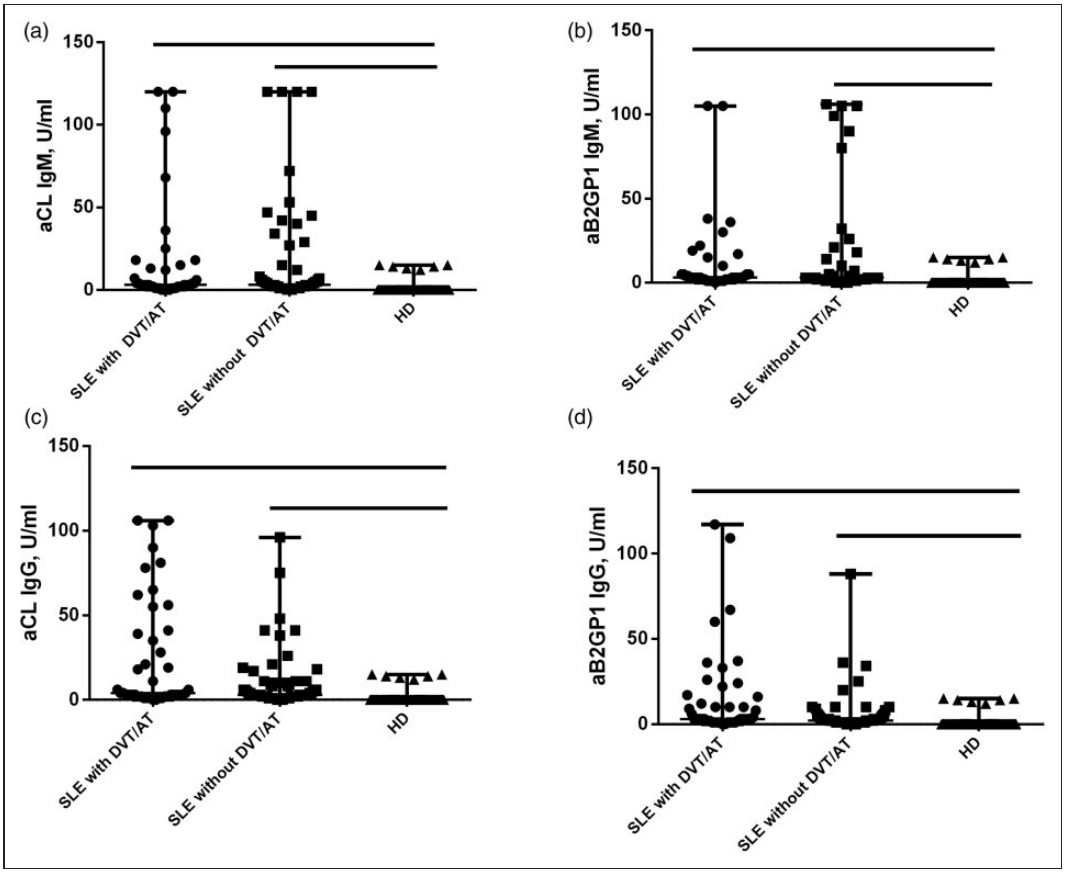

Figure 1 Comparison of quantitative anti cardiolipin (aCL) IgM (a) and anti-beta 2 glycoprotein I (aß2GPI) IgM (b), aCL IgG (c), aß2GPI IgG (d) analysis by ELISA in systemic lupus erythematosus (SLE) patients with deep vein thrombosis (DVT)/arterial thrombosis (AT) ($n = 47$), SLE without DVT/AT ($n = 60$) and healthy donors (HD) ($n = 120$).

(thrombosis, AT, DVT, recurrence of thrombotic events) and one or more independent variables including aCl, aß2GPI, aPc, aPe, aPg, aPi, aPs, aAnV, aPt IgG/IgM as well as LA, gender, age, obesity, SLEDAI and disease duration (Table 3). Positivity of IgG to Ps was an independent risk factor for thrombosis (odds ratio (OR) 3.6, 95% confidence interval (CI) 1.13, 11.34) and recurrent thrombosis (OR 6.9, 95% CI 2.08, 22.58) with age as the confounder. In contrast, aPi IgG was a risk factor for AT (OR 5.1, 95% CI 1.33, 19.83) and DVT (OR 3.9, 95% CI 1.14, 13.22) with age and obesity as confounders, respectively. Furthermore, the occurrence of more than four aPL IgG detected by LIA was an independent risk factor revealing an OR of 10.9, 95% CI 1.16, 101.54 for thrombosis with age and LA as confounders. The OR of more than four aPL was even higher for AT (OR 14.6, 95% CI 2.46, 86.27) with only age as the confounder, whereas the OR for DVT was 5.8 (95% CI 1.05, 32.42) with obesity as the confounder. For recurrent thrombosis, the OR of the occurrence of more than four aPL IgG was 35.9 (95% CI 3.76, 342.78) with age as the confounder.

When we compared the occurrence of at least two non-criteria aPLs, SLE patients with AT alone or AT with DVT more frequently demonstrated non-criteria aPL than SLE patients with DVT (12/20, 60% vs 10/32, 31.2%, $p = 0.0499$).

Discussion

The analysis of consistent aPL (>12 weeks) plays a pivotal role in the classification of APS and thus further management of patients.[18] According to the classification criteria, LA by functional coagulation assay and aCL as well as aβ2GPI by ELISA should be analysed.[1] High titers of aPLs are strongly associated with an increased risk of thromboembolism and pregnancy loss.[19] Patients with triple-positive results have a higher risk of clinical manifestation of APS.[20]

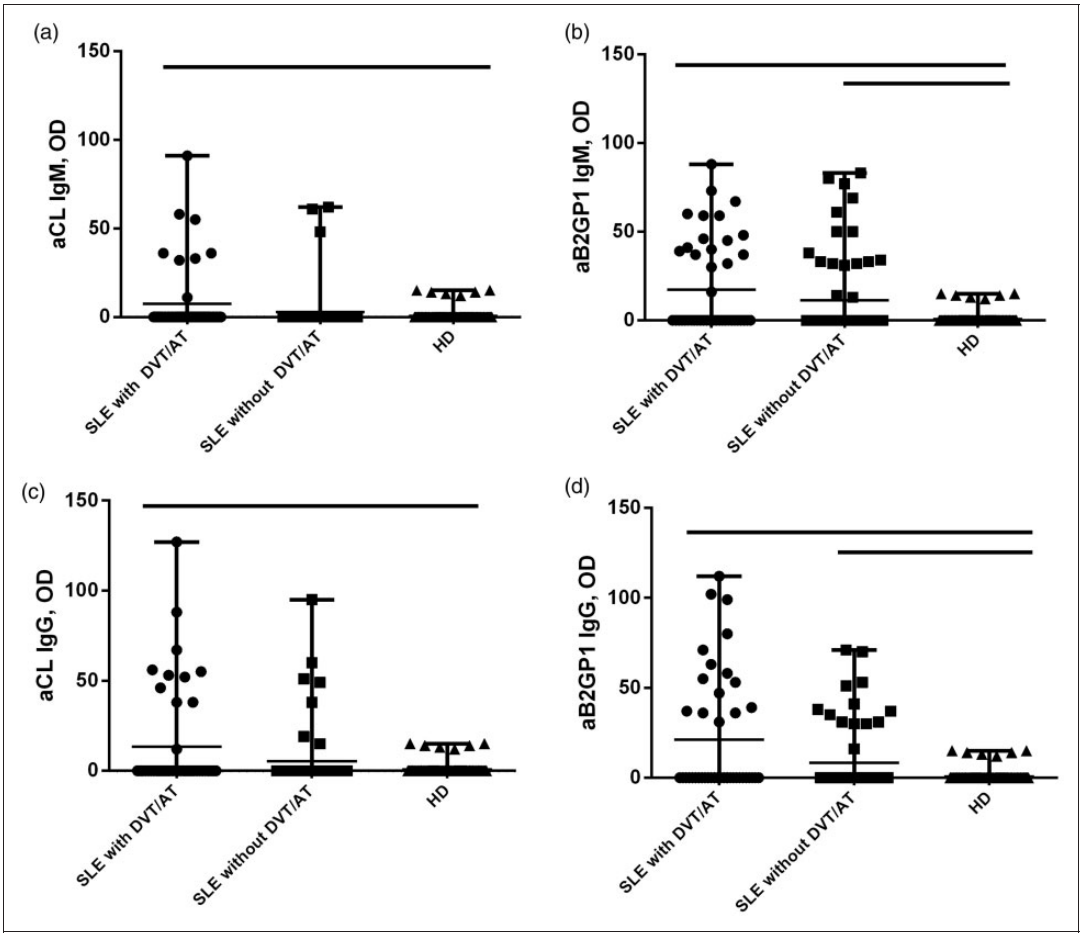

Figure 2 Comparison of quantitative anti cardiolipin (aCL) IgM (a) and anti-beta 2 glycoprotein I (aß2GPI) IgM (b), aCL IgG (c), aß2GPI IgG (d) analysis by line immunoassay (LIA) in systemic lupus erythematosus (SLE) patients with deep vein thrombosis (DVT)/ arterial thrombosis (AT) ($n = 47$), SLE without DVT/AT ($n = 60$) and healthy donors (HD) (n = 120). OD: optical density.

SLE and APS are considered closely related diseases and up to 40% of SLE patients demonstrate aPL.[21] However, given the broad spectrum of clinical manifestations of APS ranging from AT and DVT to pregnancy disorders, the association of criteria as well as non-criteria aPLs with the clinical phenotype in APS and SLE is still controversial.[13,22] In general, venous thrombosis appears more frequently associated to LA whereas coronary and peripheral artery and carotid thromboses are frequently found in patients with aCL IgG and IgM.[23]

Recently, LIA as a novel multiplex technology for aPLs detection including non-criteria aPL analysis was reported for APS serology.[10] The use of hydrophobic PVDF as a solid phase for LIA enables the oriented binding of phospholipids, which leads to a high density of negatively charged phospholipid heads on the PVDF surface. This binding appears to provide preferred conditions for the interaction with cofactors such as β2GP1 of the patient serum and, further, APS-specific aPLs through the optimal presentation of cofactor epitopes and here particularly of β2GP1 D1 epitopes.[11,24] Recently, it was reported that LIA in contrast to ELISA can be useful to discriminate patients with APS from asymptomatic carriers presumably through the preferred presentation of D1 epitopes.[12] Moreover, aPg determined by LIA along with the ratio of aD1 to aPL to domains 4 and 5 of β2GP1 analysed by ELISA differentiated APS from patients suffering from systemic rheumatic autoimmune diseases who were positive for IgG to β2GP1 but did not show APS-related symptoms.[14] Similarly, the disease specificity of the non-criteria aPg was assumed to be brought about the exposure of particular D1 epitopes after conformational changes of the patients' own β2GP1 interacting with the immobilized Pg.

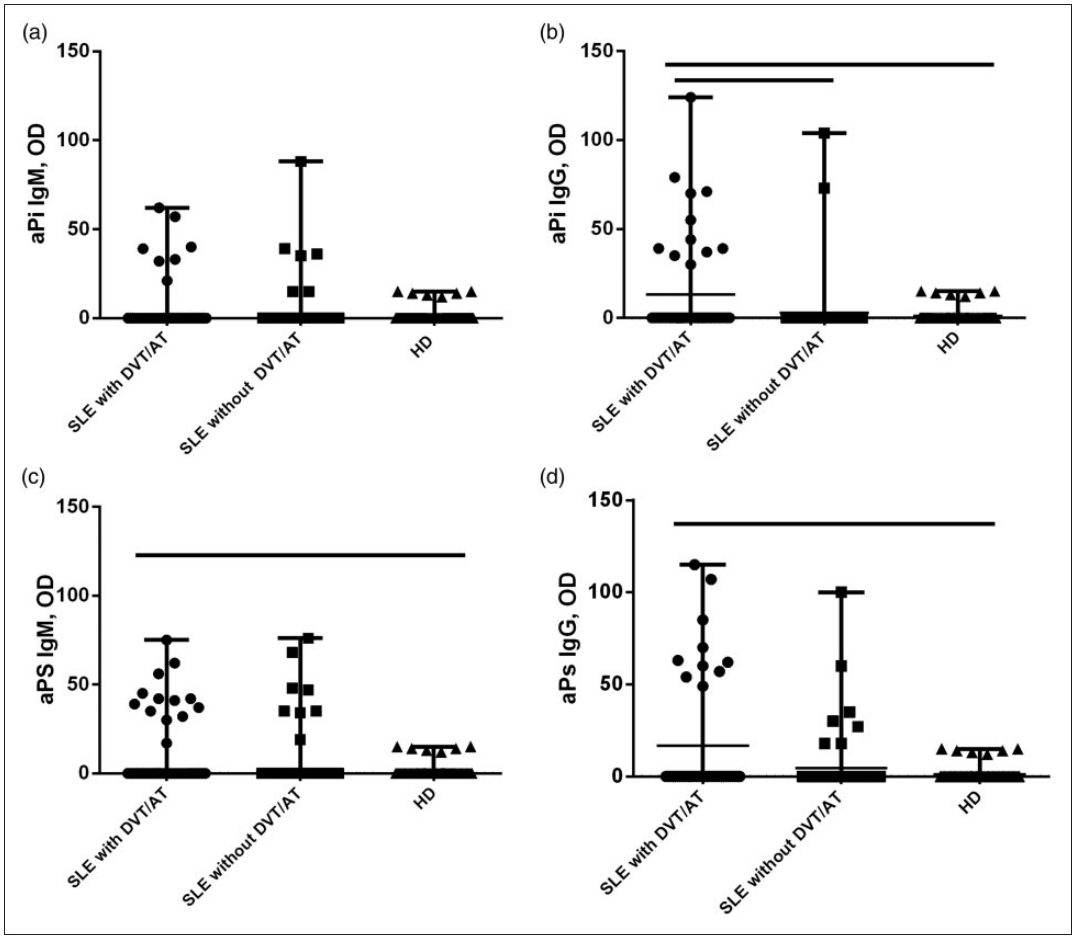

Figure 3 Comparison of quantitative antibodies to phosphatidylinositol (aPi) IgM (a) and antibodies to aPi IgG (b), antibodies to phosphatidylserine (aPs) IgM (c), aPs IgG (d) analysis detected by line immunoassay (LIA) in systemic lupus erythematosus (SLE) patients with deep vein thrombosis (DVT)/ arterial thrombosis (AT) ($n = 47$), SLE without DVT/AT ($n = 60$) and healthy donors (HD) ($n = 120$). OD: optical density.

In our study, the agreement of aPL testing by LIA and ELISA was good (aβ2GP1 IgG, aβ2GP1 IgM), moderate (aCL IgG) and fair (aCL IgM) and in line with previous reports regarding the former.[12] Quantitative criteria IgG and IgM aPL detected by ELISA and LIA were significantly correlated ($p < 0.0001$, respectively).

As reported elsewhere, LA and IgG to CL and β2GPI but not IgM detected by ELISA and LIA as well as double and triple positivity were linked with thrombosis in SLE patients in our study.[25–27] The association of double positivity with thrombosis was only significant for the combination LA/LIA. However, all criteria aPL determined by both quantitative LIA and ELISA did not reveal significantly higher levels in SLE patients with DVT/AT compared with SLE patients without thrombosis. In case of LIA, quantitative criteria aPLs levels in SLE patients without thrombosis were not significantly different to those in HD.

Non-criteria aPL to negatively charged phospholipids such as Pa, Pi and Ps or the combination of Ps with Pt have been suggested to demonstrate diagnostic and/or prognostic significance.[28] Association of aPs and aPi with pregnancy morbidity was reported.[29–31]

In our study, in SLE with DVT/AT, more than four aPLs of the IgG isotype ascertained by LIA were significantly more prevalent in contrast to SLE without DVT/AT (25% vs 4%). Furthermore, the analysis of non-criteria aPL allowed us to differentiate SLE patients with AT or AT/DVT from those with DVT alone in this study. The appearance of at least two non-criteria aPLs was significantly more frequent in SLE with AT or AT/DVT in contrast to SLE with DVT.

Moreover, we identified aPs and aPI as independent risk factors for thrombosis and recurrence thereof as well as AT and DVT, respectively. The occurrence of more than four aPL IgG detected by

Table 3 Logistic regression analysis for aPL analysed by line immunoassay in patients suffering from SLE with and without thrombosis. Significant relationships were investigated between one dichotomous dependent variable (thrombosis, AT, DVT, recurrence of thrombotic events) and one or more independent variables including (a) IgG as well as IgM to cardiolipin, beta2-glycoprotein 1, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidylserine, annexin V and prothrombin, LA, gender, age, obesity, SLEDAI and disease duration or (b) the presence of more than four positive IgG aPL as well as LA, gender, age, obesity SLEDAI and disease duration. Only significant correlations are shown (overall model fit $p < 0.05$ respectively).

	Coefficient	*Std. error*	*Odds ratio*	*95% CI*	p *value*
(a)					
Thrombosis					
aPs IgG	1.27418	0.59	3.57	1.13, 11.34	<0.05
AT					
aPi IgG	1.63649	0.69	5.13	1.33, 19.83	<0.05
Age	0.062191	0.02	1.06	1.02, 1.11	<0.001
DVT					
aPi IgG	1.35775	0.62	3.88	1.14, 13.22	<0.05
Obesity	1.00400	0.59	2.73	1.01, 7.39	<0.05
Recurrent thrombosis					
aPs IgG	1.16789	0.60	6.85	2.08, 22.58	<0.001
Age	0.041072	0.02	1.04	1.00, 1.08	<0.05
(b)					
Thrombosis					
>4 aPL IgG	2.38638	1.14	10.87	1.16, 101.54	<0.05
Age	0.035223	0.02	1.03	1.00, 1.07	<0.05
LA	1.27418	0.43	2.21	0.94, 5.21	0.07
AT					
>4 aPL IgG	2.67964	0.90	14.58	2.46, 86.27	<0.001
Age	0.067964	0.02	1.07	1.02, 1.12	<0.001
DVT					
>4 aPL IgG	1.76336	0.87	5.83	1.05, 32.42	<0.05
Obesity	0.98959	0.50	2.69	0.99, 7.25	0.05
Recurrent thrombosis					
>4 aPL IgG	3.58099	1.15	35.90	3.76, 342.78	<0.001
Age	0.050578	0.02	1.05	1.01, 1.0950	<0.05

aPL: antiphospholipid antibodies; aPi: phosphatidylinositol; aPs: phosphatidylserine; AT: arterial thrombosis; CI: confidence interval; DVT: deep vein thrombosis; LIA: line immunoassay; LA: lupus anticoagulant; SLE: systemic lupus erythematosus; SLEDAI: SLE disease activity index.

LIA was an independent risk factor for thrombosis (confounders: age, LA), arterial (confounder: age) and venous (confounder: obesity) thrombosis as well as the recurrence of thrombosis (confounder: age) with remarkably high OR. Thus, the OR of the presence of more than four IgG aPL reached a value of 36.9 (95% CI 3.76, 342.78) for recurrence of thrombosis. Of note, LA was only an independent risk for thrombosis with an OR of 2.2 (95% CI 0.94, 5.21). Disease duration and activity as well as gender and IgM aPL did not show a relation with thrombotic events and their recurrence in SLE. This lends further credit to the assumption that aPL IgG play a pathogenic role for thrombotic events in SLE and recurrence of thrombosis is associated with the occurrence of multiple aPL. Of note, we did not find any association of aPL with obstetric complications in this study.

In summary, LIA is a promising multiplex test for the simultaneous detection of criteria and non-criteria aPLs. Profiling of aPLs detected by LIA can aid in the differentiation of SLE with thrombosis from SLE without and in particular of SLE with AT from SLE with DVT alone. aPL profiling by LIA can aid in the risk assessment of thrombosis and its recurrence.

Declaration of conflicting interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: DR has a management role and is a shareholder of GA Generic Assays GmbH and Medipan GmbH. Both companies are diagnostic manufacturers. The remaining authors have no conflicts of interest to declare.

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ORCID iD

O Tkachenko http://orcid.org/0000-0002-1479-6551

References

1 Miyakis S, Lockshin MD, Atsumi T, *et al*. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J Thromb Haemost* 2006; 4: 295–306.
2 Gómez-Puerta JA, Cervera R. Diagnosis and classification of the antiphospholipid syndrome. *J Autoimmun* 2014; 48–49: 20–25.

3 Meroni PL, Borghi MO, Raschi E, Tedesco F. Pathogenesis of antiphospholipid syndrome: Understanding the antibodies. *Nat Rev Rheumatol* 2011; 7: 330–339.
4 Asherson RA, Cervera R. Antiphospholipid antibodies and infections. *Annals Rheu Dise* 2003; 62: 388–393.
5 Quéméneur T, Lambert M, Hachulla E, *et al*. Significance of persistent antiphospholipid antibodies in the elderly. *J Rheumatol* 2006; 33: 1559–1562.
6 Versini M. Thyroid autoimmunity and antiphospholipid syndrome: Not such a trivial association. *Front Endocrinol (Lausanne)* 2017; 8: 175.
7 Ho KT, Reveille JD. The clinical relevance of autoantibodies in scleroderma. *Arthritis Res Ther* 2003; 5: 80–93.
8 de Groot P, Urbanus R. The future of antiphospholipid antibody testing. *Semin Thromb Hemost* 2012; 38: 412–420.
9 Misasi R, Capozzi A, Longo A, *et al*. 'New' Antigenic targets and methodological approaches for refining laboratory diagnosis of antiphospholipid syndrome. *J Immunol Res* 2015; 2015: 1–13.
10 Roggenbuck D, Egerer K, von Landenberg P, *et al*. Antiphospholipid antibody profiling: Time for a new technical approach? *Autoimmun Rev* 2012; 11: 821–826.
11 Egerer K, Roggenbuck D, Büttner T, *et al*. Single-step autoantibody profiling in antiphospholipid syndrome using a multi-line dot assay. *Arthritis Res Ther* 2011; 13: R118.
12 Roggenbuck D, Borghi MO, Somma V, *et al*. Antiphospholipid antibodies detected by line immunoassay differentiate among patients with antiphospholipid syndrome, with infections and asymptomatic carriers. *Arthritis Res Ther* 2016; 18: 111.
13 Belizna C, Stojanovich L, Cohen-Tervaert JW, *et al*. Primary antiphospholipid syndrome and antiphospholipid syndrome associated to systemic lupus: Are they different entities? *Autoimmun Rev* 2018; 17: 739–745.
14 Nalli C, Somma V, Andreoli L, *et al*. Anti-phospholipid IgG antibodies detected by line immunoassay differentiate patients with anti-phospholipid syndrome and other autoimmune diseases. *Autoimmun Highlights* 2018; 9: 6.
15 Petri M, Orbai A-M, Alarcón GS, *et al*. Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. *Arthritis Rheum* 2012; 64: 2677–2686.
16 Devreese KMJ. Antiphospholipid antibody testing and standardization. *Int J Lab Hematol* 2014; 36: 352–363.
17 Moore G. Recent guidelines and recommendations for laboratory detection of lupus anticoagulants. *Semin Thromb Hemost* 2014; 40: 163–171.
18 Tektonidou MG, Andreoli L, Limper M, *et al*. EULAR recommendations for the management of antiphospholipid syndrome in adults. *Ann Rheum Dis* 2019; 78: 1296–1304.
19 Pengo V, Banzato A, Denas G, *et al*. Correct laboratory approach to APS diagnosis and monitoring. *Autoimmun Rev* 2013; 12: 832–834.
20 Pengo V, Biasiolo A, Pegoraro C, *et al*. Antibody profiles for the diagnosis of antiphospholipid syndrome. *Thromb Haemost* 2005; 93: 1147–1152.
21 Tincani A, Andreoli L, Chighizola C, *et al*. The interplay between the antiphospholipid syndrome and systemic lupus erythematosus. *Autoimmunity* 2009; 42: 257–259.
22 Meroni PL, Tsokos GC. Editorial: Systemic lupus erythematosus and antiphospholipid syndrome. *Front Immunol* 10. Epub ahead of print 25 Februay 2019. DOI: 10.3389/fimmu.2019.00199.
23 Boura P, Tselios K, Skendros P, *et al*. Antiphospholipid syndrome in Greece: Clinical and immunological study and review of the literature. *Angiol* 2004; 55: 421–430.
24 Roggenbuck D, Somma V, Schierack P, *et al*. Autoantibody profiling in APS. *Lupus* 2014; 23: 1262–1264.
25 Kelchtermans H, Pelkmans L, de Laat B, *et al*. IgG/IgM antiphospholipid antibodies present in the classification criteria for the antiphospholipid syndrome: A critical review of their association with thrombosis. *J Thromb Haemost* 2016; 14: 1530–1548.
26 Reynaud Q, Lega J-C, Mismetti P, *et al*. Risk of venous and arterial thrombosis according to type of antiphospholipid antibodies in adults without systemic lupus erythematosus: A systematic review and meta-analysis. *Autoimmun Rev* 2014; 13: 595–608.
27 Galli M, Luciani D, Bertolini G, *et al*. Lupus anticoagulants are stronger risk factors for thrombosis than anticardiolipin antibodies in the antiphospholipid syndrome: A systematic review of the literature. *Blood* 2003; 101: 1827–1832.
28 Hoxha A, Ruffatti A, Mattia E, *et al*. Relationship between antiphosphatidylserine/prothrombin and conventional antiphospholipid antibodies in primary antiphospholipid syndrome. *Clin Chem Lab Med;* 53. Epub ahead of print 1 January 2015. DOI: 10.1515/cclm-2014-1129.
29 Rote NS, Johnson DD, Ware Branch D. Antiphospholipid antibodies and recurrent pregnancy loss: Correlation between the activated partial thromboplastin time and antibodies against phosphatidylserine and cardiolipin. *Am J Obstet Gynecol* 1990; 163: 575–584.
30 Ziporen L, Blank M, Shoenfeld Y. Animal models for antiphospholipid syndrome in pregnancy. *Rheum Dis Clin North Am* 1997; 23: 99–117.
31 Ulcova-Gallova Z, Krauz V, Novakova P, *et al*. Anti-phospholipid antibodies against phosphatidylinositol, and phosphatidylserine are more significant in reproductive failure than antibodies against cardiolipin only. *Am J Reprod Immunol*. Epub ahead of print 2005. DOI: 10.1111/j.1600-0897.2005.00294.x.