RHEUMATOLOGY

Rheumatology 2013;52:501–509
doi:10.1093/rheumatology/kes252
Advance Access publication 18 November 2012

Original article

Clinical manifestations and anti-phospholipid antibodies in 712 patients with systemic lupus erythematosus: evaluation of two diagnostic assays

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Abstract

Objectives. To evaluate the agreement and performance of two tests for aPLs with regard to association with manifestations of the APS in patients with SLE.

Methods. We investigated 712 SLE patients and 280 population controls. Cardiolipin and β_2 glycoprotein-I antibodies were measured with routine ELISA and a new automated method. Three positivity cut-offs (99%, 90% of controls and recommended cut-off by manufacturers) were used. Associations with previous thrombotic events, thrombocytopenia and, in a subgroup of patients, obstetric morbidity ($n = 296$) were evaluated. Results were compared with the LA test, performed in 380 patients.

Results. Inter-test agreement was moderate (demonstrated by κ-values 0.16–0.71). Performance of the two tests was similar: at the 99th percentile cut-off, sensitivity for any thrombotic event ranged from 3.7% to 24.8%, while specificity was 84.7–97.7%. Regardless of assay, IgG isotypes were associated with venous thrombosis and ischaemic cerebrovascular disease, whereas aPLs of IgM isotype were weakly associated with ischaemic heart disease. Associations were greatly affected by aPL level. LA performed better than the specific aPL tests. LA was associated with any thrombotic event, odds ratio 5.4 (95% CI 3.1, 9.4), while the specific aPL tests ranged from non-significant to an odds ratio of 1.9 (95% CI 1.03, 3.4) using criteria cut-off. LA was also convincingly associated with other APS manifestations.

Conclusion. In relation to thrombotic manifestations, there was moderate agreement but no clear advantages when comparing a routine aPL ELISA with an automated method. APL isotype and titre as well as LA positivity are important for risk assessment in SLE patients.

Key words: anticardiolipin antibodies, β_2 glycoprotein-I antibodies, lupus anticoagulant, isotype, titre, ELISA, systemic lupus erythematosus, thrombosis, cardiovascular disease.

CLINICAL SCIENCE

Introduction

There are several problems with the current methods for detection of aPLs on which the diagnosis of APS depend [1, 2]. From a practical view, the currently used ELISAs are relatively time-, money- and manpower-intensive and standardization is poor [3]. Possible replacement methods could be more automated immunoassays. However, independent publications on the performance of such methods are scarce [4, 5]. This stresses the need to conduct additional studies in representative patient settings before decisions on method switching.

Another key issue is the interpretation of the aPL tests in everyday practice. One important clinical question is whether it is only high titres (which are associated with multi-test positivity) that are linked to increased risk for thrombosis. Results from previous studies are conflicting [6–18]. A second clinical question is whether there is a

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Submitted 19 March 2012; revised version accepted 7 August 2012.

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need for the whole panel of currently used aPL tests. From earlier publications, some tests (especially the LA) and aPL isotypes (IgG > IgM) appear to be more important than others [3, 19–21]. However, the relevance of different aPL tests seems to differ according to the APS manifestation and the population studied. Sometimes no associations whatsoever are found [22].

To shed light on the issues presented above, we studied two different aPL tests in a large population of patients with confirmed diagnosis of SLE and in matched population controls [2]. First, we investigated the inter-test agreement and the individual performances of our routinely used ELISA test for aPL and a new, fully automated method. Secondly, we examined the associations between these aPL tests and prior APS manifestations [2] among SLE patients with a focus on thrombosis. Thirdly, we studied the performance of different cut-offs for positivity with respect to previous thrombotic events.

Materials and methods

Study population

We included 712 patients clinically diagnosed with SLE from the rheumatology clinics at Karolinska, Uppsala and Lund University Hospitals ($n = 381$, $n = 139$ and $n = 192$, respectively). For a clinical diagnosis of SLE we required characteristic SLE serology in combination with, as a minimum, two typical organ manifestations and the absence of more plausible explanations for the condition [23]. The great majority of the patients also fulfilled at least four of the 1982 revised classification criteria for SLE according to the ACR, but 25 patients fulfilled fewer than four criteria [24]. At inclusion, information about previous thrombotic events was collected through interviewing the patients and medical records. Furthermore, blood samples for serological and biochemical analyses were drawn. For the Karolinska patients, data on obstetric morbidity were also collected.

To calculate a cut-off level corresponding to the 99th and 90th percentile we used samples from 280 controls from the general population without any history of thrombosis or obstetric morbidity, as defined in the APS criteria [2]. These individuals were matched to the Karolinska patients for age, sex and region of living. Informed consent from study subjects and approval from the local ethics committees (KI Forskningsetikkommitté Nord, Karolinska sjukhuset; Regionala etikprövningsnämnden i Stockholm; Forskningsetikkommittén Akademiska sjukhuset/University Hospital and Forskningsetikkommittén i Lund/Malmö) were obtained.

Definition of clinical events

Clinical APS manifestations studied were the following objectively verified vascular events:

(i) Ischaemic heart disease (IHD): myocardial infarction (MI), confirmed by electrocardiography and a rise in plasma creatine kinase, muscle and brain fraction (CK-MB) or troponine T and/or angina pectoris confirmed by exercise stress test.
(ii) Ischaemic cerebrovascular disease (ICVD): stroke including cerebral infarction, confirmed by CT or MRI and/or transitory ischaemic attacks (TIAs), defined as transient focal symptoms from the brain or retina with a maximum duration of 24 h.
(iii) Ischaemic peripheral vascular disease (IPVD): intermittent claudication and/or peripheral arterial thrombosis or embolus confirmed by angiogram or Doppler flow studies.
(iv) Venous thromboembolism (VTE): defined as deep vein thrombosis confirmed by venography or ultrasonography and/or pulmonary embolism, confirmed by pulmonary perfusion scintigram, angiogram or spiral CT.

The term any arterial event (AT) refers to the occurrence of $\geqslant 1$ of events (i)–(iii). The term any thrombotic event (ATE) refers to the occurrence of $\geqslant 1$ of the events (i)–(iv).

We also tabulated obstetric morbidity, defined according to established APS criteria [2], and thrombocytopenia, defined according to ACR criteria [24].

Tests for aPL

Antibodies against cardiolipin of IgG and IgM isotype (aCL IgG and aCL IgM) as well as antibodies against β_2 glycoprotein-I of IgG isotype (anti-β_2GPI IgG) were analysed by a routinely performed ELISA (Orgentec, Mainz, Germany) and a new, fully automated fluorescence enzyme immunoassay method (Elia Cardiolipin IgG, Elia Cardiolipin IgM, Elia β_2 GPI IgG performed on Phadia 250, Phadia AB, now Thermo Fisher Scientific, Germany), according to the manufacturer's instructions. With the latter method, antibodies against β_2 GPI of IgM isotype (anti-β_2GPI IgM) were also analysed (Elia β_2GPI). The two methods are from now on referred to as the routine ELISA method (RM) and the automated method (AM).

The manufacturer's recommended cut-offs were set at the 99th percentile of blood donors for RM and corresponded to the 95th percentile for aCL and to at least the 97th percentile for anti-β_2GPI for AM. The aCL assays were calibrated to Harris standards. RM aCL and anti-β_2GPI assays had also tested positive to HCAL/EY2C9, Harris and IRP 97/656 (IgG) according to the product information (www.orgentec.com, Product Information Elia Cardiolopin).

The intra- and inter-assay coefficients of variation (CV%) obtained from the manufacturer's product information were for RM: aCL IgG 2.5–5.8%, aCL IgM 2.5–5.3% and anti-β_2GPI IgG 2.6–7.9% and for AM: aCL IgG 2.4–3.9%, aCL IgM 3.2–5.4%, anti-β_2GPI IgG 3.0–4.7% and anti-β_2GPI IgM 1.9–4.3%. Experience at our laboratory from routine performance of RM resulted in a higher CV% between 13.5 and 20% (unpublished data).

The presence or absence of LA was determined with a modified Dilute Russel Viper Venom method (Biopool, Umeå, Sweden) using Bioclot LA according to standard procedure. Patient samples were diluted 1:1 with normal plasma and clotting time was measured with the addition of the LA reagent and compared with the clotting time for normal plasma. As a confirmation test, concentrations of

phospholipids were increased: LA was considered positive if normalization of clotting time was achieved. Platelets were measured according to RMs.

Statistical analysis

Agreement and performance of the two methods for aPL testing

As a measure of agreement between tests (aCL IgG, aCL IgM and anti-β_2GPI IgG from both manufacturers) we calculated the κ-coefficient for a cut-off corresponding to the 99th and 90th percentiles of our controls, who were free of clinical APS manifestations [2], and the cut-off corresponding to the manufacturer's recommendation.

To compare the performance of the two tests in the clinical setting of SLE patients, sensitivity, specificity and positive and negative predictive value (PPV and NPV, respectively) were calculated using a cut-off corresponding to the 99th and 90th percentiles of our controls.

Association between different aPL and thrombotic manifestations and the impact of different cut-offs for positivity

Contingency tables and calculations of odds ratios (ORs) with 95% CIs were used to analyse associations between nominal variables. ORs were calculated both for a cut-off corresponding to the 99th and 90th percentiles of our controls and for a cut-off corresponding to the manufacturer's recommendation.

For the Karolinska patients, samples were analysed in routine care and continuous values were only registered above the recommended cut-off by the manufacturer. Thus data below this level were not reported. In the analyses requiring full continuous data we therefore only included the 331 patients from Uppsala and Lund (for RM by using a curve approximation to estimate the low titres). Values below what is normally considered the detection limit ($\sim$1 for all tests), and thus not reliable, were given a value of 0.9.

Comparison between the specific aPL tests and the LA test regarding association with thrombosis

Sensitivity, specificity, PPV, NPV and OR were calculated for the LA test, performed in 380 Karolinska patients, as a comparison.

Associations between aPL tests and other clinical features of APS

ORs for obstetric morbidity and thrombocytopenia were calculated for a cut-off corresponding to the manufacturer's recommendation for the different aPL tests and LA, respectively.

Statistical analysis was performed using JMP software (SAS Institute, Cary, NC, USA). A P-value $\leqslant 0.05$ was considered to be statistically significant.

Results

Basic characteristics of patients and controls are presented in Table 1. For the 520 patients from Karolinska and Uppsala, data on current medication were available: 56% were treated with steroids and 15% with warfarin (of the 380 patients tested for LA, 16% were on warfarin treatment).

Table 1 Clinical characteristics of the 712 patients with SLE and 280 controls

	SLE patients	Controls
Gender, females, number (%)	633/712 (88.9)	259/280 (92.5)
Age, mean (S.D.), years	49.2 (15.5)	46.4 (14.8)
Age at disease onset, mean (S.D.), years	34.4 (14.7)	0
Disease duration, mean (S.D.), years	14.8 (11.1)	0
Number of ACR criteria, mean (S.D.)[a]	5.7 (1.5)	NR
Malar rash, number of patients (%)	397 (55.8)	NR
Discoid rash, number of patients (%)	166 (23.3)	NR
Photosensitivity, number of patients (%)	488 (68.5)	NR
Oral ulcers, number of patients (%)	205 (28.8)	NR
Arthritis, number of patients (%)	565 (79.3)	NR
Serositis, number of patients (%)	302 (42.4)	NR
Nephritis, number of patients (%)	237 (33.3)	NR
Neurology, number of patients (%)	74 (10.4)	NR
Haematology, number of patients (%)	463 (65.0)	NR
Immunology, number of patients (%)	499 (70.0)	NR
ANA, number of patients (%)	698 (98.0)	NR
Thrombocytopenia, number of patients (%)	142 (20.0)	NR
Any thrombosis (venous or arterial), number of patients (%)	223 (31.3)	0
Any arterial thrombosis, number of patients (%)	150 (21.1)	0
IHD, number of patients (%)	73 (10.3)	0
ICVD, number of patients (%)	74 (10.4)	0
Venous thrombosis, number of patients (%)	117 (16.4)	0

[a]ACR criteria are the criteria for SLE according to the ACR: the criteria listed below this and thrombocytopenia refer to these criteria [24]. NR: not reported.

Agreement and performance of the two methods for aPL testing

Calculated cut-offs corresponding to the 99th and 90th percentiles and the manufacturer's recommendation are presented in Table 2. In general the cut-offs recommended by the manufacturer were higher than the 90th percentile cut-off but lower than the 99th percentile cut-off of our controls.

Data from the 331 patients with continuous values were used to calculate the κ-coefficients for cut-offs corresponding to the 99th and 90th percentiles, while all 712 patients were included in the calculations of the κ-coefficient using a cut-off corresponding to the manufacturer's recommendation. The κ-values ranged from 0.16 to 0.71 (Fig. 1). For aCL IgG, aCL IgM and anti-β_2GPI IgG, agreement between the two methods were best using the cut-offs corresponding to the manufacturer's recommendation. The κ-values were lower using both the generally lower cut-offs corresponding to the 90th percentile and the higher cut-offs corresponding to the 99th percentile. The lowest κ-value was calculated for the 90th percentile cut-off of aCL IgM.

Sensitivity, specificity, PPV and NPV for any thrombotic event employing a cut-off corresponding to the 99th and 90th percentiles ($n = 331$) are shown in Table 3.

Association between different aPL and thrombotic manifestations and the impact of different cut-offs for positivity

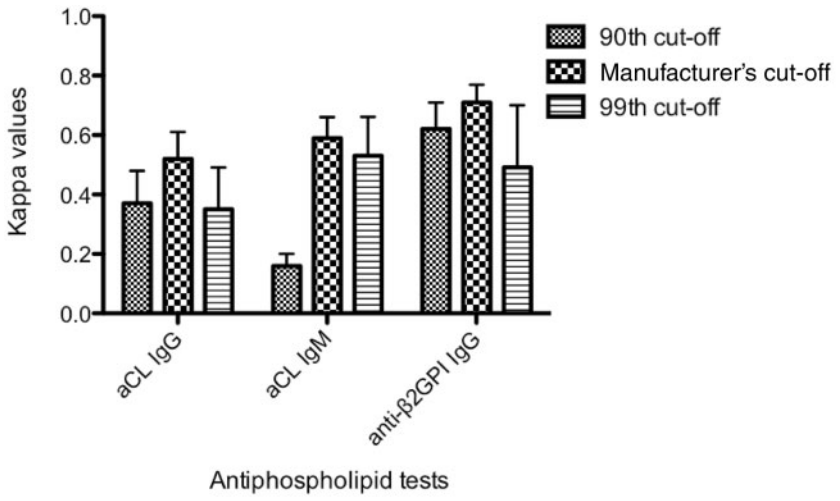
ORs for the associations with thrombotic events using cut-offs corresponding to the 99th and 90th percentiles ($n = 331$) and the manufacturer's recommendation

Table 2 Three cut-off levels for routine and automated aPL assays and the number of positive patients at these cut-offs

aPL	Cut-off level, 99th percentile	Cut-off level, 90th percentile	Cut-off level, manufacturer	Number (%) of positive, 99th percentile ($n = 331$)	Number (%) of positive, 90th percentile ($n = 331$)	Number (%) of positive, manufacturer ($n = 712$)
aCL IgG (RM)	9	5	10	53 (16.0)	94 (28.4)	122 (17.1)
aCL IgG (AM)	30	7	10	17 (5.1)	78 (23.6)	117 (16.4)
Anti-β_2GPI IgG (RM)	15	4	5	26 (7.9)	85 (25.7)	144 (20.2)
Anti-β_2GPI IgG (AM)	53	3	7	9 (2.7)	93 (28.0)	117 (16.4)
aCL IgM (RM)	8	3	7	61 (18.4)	252 (76.1)	149 (20.9)
aCL IgM (AM)	30	8	10	28 (8.5)	77 (23.3)	146 (20.5)
Anti-β_2GPI IgM (AM)	12	2	7	32 (9.7)	113 (34.1)	103 (14.5)

The cut-offs corresponding to the 99th and 90th percentiles of controls and the manufacturer's recommendation (three left columns) with the corresponding number of positive patients (three right columns) are given. The two most important cut-offs are those of the 99th percentile and the manufacturer's recommendation (second and fourth columns).

Fig. 1 Agreement between the corresponding RM and AM at different cut-offs for positivity.



Agreement expressed as κ-values for different aPL tests (with 95% CI) comparing the RM and AM for cut-offs corresponding to the 99th and 90th percentiles of controls ($n = 331$) and manufacturer's recommendation ($n = 712$).

TABLE 3 Performance of aPL tests at different cut-off levels with respect to any previous thrombotic event

aPL	Sensitivity (%), 99th percentile cut-off	Specificity (%), 99th percentile cut-off	PPV (%), 99th percentile cut-off	NPV (%), 99th percentile cut-off	Sensitivity (%), 90th percentile cut-off	Specificity (%), 90th percentile cut-off	PPV (%), 90th percentile cut-off	NPV (%), 90th percentile cut-off
aCL IgG (RM)	22.0	86.9	45.3	69.4	40.4	77.5	46.8	72.6
aCL IgG (AM)	5.5	95.0	35.3	67.2	31.2	80.2	43.6	70.4
anti-β_2GPI IgG (RM)	11.0	93.7	46.2	68.2	34.9	78.8	44.7	71.1
anti-β_2GPI IgG (AM)	3.7	97.7	44.4	67.4	34.9	75.2	40.9	70.2
aCL IgM (RM)	24.8	84.7	44.3	69.6	78.9	25.2	34.1	70.9
aCL IgM (AM)	11.9	93.2	46.4	68.3	29.4	79.7	41.6	69.7
anti-β_2GPI IgM (AM)	11.0	91.0	37.5	67.6	45.0	71.2	43.4	72.5

Three hundred and thirty-one patients with SLE are included in these calculations. Cut-offs are based on the 99th percentile and 90th percentile of controls (free of clinical APS manifestations). The most important results are sensitivity and specificity at the 99th percentile cut-off (columns 2 and 3).

($n = 712$) for single positive aPL tests as well as for double positivity are presented in Table 4. Double positivity was defined as two positive tests of either aCL IgG, aCL IgM or an anti-β_2GPI IgG from the same manufacturer.

Comparison between the specific aPL tests and the LA test regarding association with thrombosis

For comparison, the ORs for LA using data from the 380 Karolinska patients are also demonstrated in Table 4. When we added a positive LA test to double positivity using the manufacturer's recommended cut-off, the OR (95% CI) for any thrombosis increased from 2.5 (1.7, 3.7) to 5.4 (3.1, 9.4) for RM. In contrast, when adding a positive LA test to double positivity by the AM according to the manufacturer's recommended cut-off, the OR (95% CI) for any thrombosis remained approximately the same: 2.6 (1.4, 4.7).

For the LA test, sensitivity for any previous thrombosis was 36.8%, whereas specificity, PPV and NPV were 90.2, 61.8 and 76.9%, respectively. Sixty-eight (17.9%) patients were positive for LA.

Associations between aPL tests and other clinical features of APS

For 296 female patients from Karolinska, data on obstetric morbidity as defined in the APS criteria [2] were accessible: 50 (17%) patients had experienced obstetric events. Of the aPLs measured, only aCL IgG (RM) and LA were significantly associated with obstetric APS manifestations: OR 2.3 (95% CI 1.2, 4.7) and 2.2 (1.1, 4.4), respectively.

Of the 712 SLE patients, 142 presented with thrombocytopenia. aCL IgG (RM), anti-β_2GPI IgG (RM), anti-β_2GPI IgG (AM) and LA were associated with thrombocytopenia: OR (95% CI) 1.8 (1.2, 2.8), 1.6 (1.0, 2.5), 1.8 (1.1, 2.8) and 2.4 (1.4, 4.4), respectively.

Discussion

In this large group of SLE patients, we report only a moderate agreement between our RM and a new automated aPL method using a cut-off corresponding to the 99th percentile of non-thrombotic controls as stated in the Sydney criteria [2]. Agreement was improved when using the generally lower cut-offs suggested by the manufacturer. Despite these discrepancies, the performance of the two investigated assays was approximately equal with respect to association with previous thrombosis. Overall, the associations with thrombosis were modest, and this was also true for the associations with obstetric morbidity and thrombocytopenia investigated as a complementary analysis. ORs for the LA tests were generally higher than for both investigated assays. However, an interesting isotype pattern was observed for the specific aPL assays.

The presented results are of interest for several reasons. First, we are one of the first groups to conduct a comparative study between a new, fully automated aPL method and currently used routine ELISAs. We report that the investigated tests show no more than moderate agreement for aPL detection, but conclude, in accordance

Table 4 Associations between the aPL tests and various thrombotic events, using different cut-offs for positivity

Test	Any thrombosis	Arterial thrombosis	IHD	ICVD	Venous thrombosis
aCL IgG (RM)					
99th percentile	1.9 (1.03, 3.4)	NS	NS	NS	3.1 (1.6, 6.0)
90th percentile	2.3 (1.4, 3.8)	NS	NS	NS	2.9 (1.6, 5.1)
Manufacturer	2.3 (1.6, 3.5)	1.6 (1.01, 2.5)	NS	2.1 (1.2, 3.6)	2.6 (1.7, 4.1)
aCL IgG (AM)					
99th percentile	NS	NS	NS	NS	NS
90th percentile	1.8 (1.1, 3.1)	NS	NS	NS	3.1 (1.7, 5.6)
Manufacturer	2.2 (1.4, 3.2)	1.6 (1.02, 2.5)	NS	2.2 (1.3, 3.9)	2.7 (1.7, 4.2)
anti-β_2GPI IgG (RM)					
99th percentile	NS	NS	NS	2.9 (1.1, 7.7)	2.6 (1.1, 6.1)
90th percentile	2.0 (1.2, 3.3)	NS	NS	NS	2.7 (1.5, 4.8)
Manufacturer	2.2 (1.5, 3.2)	1.7 (1.1, 2.5)	NS	2.1 (1.2, 3.5)	2.6 (1.7, 4.0)
anti-β_2GPI IgG (AM)					
99th percentile	NS	NS	NS	NS	NS
90th percentile	NS	NS	NS	NS	2.5 (1.4, 4.4)
Manufacturer	2.1 (1.4, 3.1)	1.6 (1.02, 2.5)	NS	2.6 (1.5, 4.5)	2.1 (1.3, 3.4)
aCL IgM (RM)					
99th percentile	1.8 (1.03, 3.2)	NS	NS	NS	NS
90th percentile	NS	NS	NS	NS	NS
Manufacturer	1.7 (1.2, 2.5)	1.7 (1.1, 2.6)	1.7 (1.02, 3.0)	NS	NS
aCL IgM (AM)					
99th percentile	NS	2.4 (1.05, 5.3)	2.7 (1.002, 7.2)	NS	NS
90th percentile	NS	NS	2.3 (1.1, 4.7)	NS	NS
Manufacturer	NS	1.6 (1.03, 2.4)	NS	NS	NS
anti-β_2GPI IgM (AM)					
99th percentile	NS	NS	NS	NS	NS
90th percentile	2.0 (1.3, 3.2)	2.1 (1.2, 3.5)	4.8 (2.2, 10.3)	NS	NS
Manufacturer	1.6 (1.06, 2.5)	1.7 (1.05, 2.7)	NS	NS	NS
Double positivity (RM)					
99th percentile	2.0 (1.01, 4.0)	NS	NS	NS	2.7 (1.3, 5.6)
90th percentile	2.4 (1.5, 3.9)	1.8 (1.1, 3.1)	NS	NS	2.6 (1.5, 4.6)
Manufacturer	2.5 (1.7, 3.7)	1.8 (1.2, 2.8)	NS	2.4 (1.4, 4.1)	2.9(1.8, 4.5)
Double positivity (AM)					
99th percentile	NS	NS	NS	NS	NS
90th percentile	2.2 (1.3, 3.7)	NS	NS	NS	2.5 (1.4, 4.6)
Manufacturer	2.6 (1.7, 4.0)	2.0 (1.3, 3.3)	NS	3.0 (1.7, 5.3)	2.6(1.6, 4.3)
LA	5.4 (3.1, 9.4)	3.1 (1.7, 5.6)	NS	5.0 (2.5, 10.0)	4.3 (2.3, 8.0)

All significant OR (95% CI) for thrombotic manifestations using RM and AM, respectively, with cut-offs corresponding to 99th and 90th percentiles of controls ($n = 331$) and manufacturer's recommendation ($n = 712$) as well as OR for LA ($n = 380$) are reported. NS: non-significant.

with previous studies [4, 5], that levels of performance in a clinically relevant setting are mostly comparable. An important reason for this inconsistency is probably the overall modest association between thrombosis and a single positive aPL test, also reported by other groups [6, 12]. This finding stresses the need for the functional LA test, which in previous studies performs better than the specific aPL tests [19] and did so also in our study (although the comparison was hampered by the fact that the test was only carried out in a subpopulation of patients). It also underlines the importance of considering outcomes from multiple tests [12] as well as other cardiovascular risk factors [17, 25] when estimating the thrombotic risk for individual SLE patients.

Secondly, our results illustrate the different strengths of the individual aPL tests and the rational for keeping them in our current standard panel. When we split the thrombotic events into subgroups, IgM antibodies were the only aPL associated with IHD, while IgG antibodies were linked to ICVD and VTE. This isotype pattern of association was suggested in a previous genetic study [26]. The association between IHD and IgM antibodies is weak, but it was replicated with assays from both manufacturers. Though the connection between aPL of IgG isotype and ICVD/VTE is well established [19, 26–28], positive relationships between aPL of IgM isotype and IHD are, with some rare exceptions [26, 29], commonly not found [14, 27, 28, 30]. We believe that the observed isotype pattern may be difficult to detect in smaller studies, when any thrombosis or arterial thrombosis is analysed as a group [8, 13, 21, 25, 31] or when antibodies are not subdivided according to isotype [8, 15, 19, 32–34]. Even though the exact

underlying mechanisms are unclear, it is possible that the observed isotype pattern indicates important aetiological differences behind different types of thrombotic events and could justify further use of aPL IgM tests.

Thirdly, our study adds valuable knowledge about the impact of different cut-off levels for positivity. We examined how three different cut-offs performed in relation to previous thrombotic events. In most cases the cut-off suggested by the manufacturer was greater than the 90th percentile but lower than the 99th percentile of our controls (cut-off according to established criteria) [2]. We conclude that aPL titres lower than the criteria cut-off were not without importance. The lack of complete results for LA made it impossible to fully sub-classify the patients according to recommended criteria [2]. However, we did examine the effect of double positivity and found that, in particular, double positivities at titres lower than the criteria were associated with previous thrombosis. A similar trend was also seen for the individual aPL tests. This is probably a question of statistical power but could be of interest for clinicians handling SLE patients. We have previously reported that low aPL titres are predictive of the first AT in a prospective study of SLE patients [17]. It is possible that the cut-off used for risk assessment in lupus patients should be lower than for defining and diagnosing primary APS patients.

Strengths of this study are the large and well-characterized SLE population and the two aPL assays, which were both performed in the same laboratory on blood samples collected at the same point in time. However, an important limitation is the cross-sectional study design relating the results of a single antibody test with a history of thrombotic events. Thus it is not clear how many of the patients that actually fulfil the Sydney criteria for APS [2], with its requirement of persistent aPL positivity and timing with clinical events. Potential interpretation problems arise since antibody titres could decrease or increase over time; e.g. due to infections [35], thrombotic risk in SLE patients could be affected by therapeutic measures taken as a consequence of previous positive aPL tests and only survivors of thrombotic events can be evaluated. Thus from this study we cannot draw any firm conclusions on the association between having a positive test before the event and the actual thrombosis, i.e. the predictive value of the investigated tests. Associations could easily be both over and underestimated. Another limitation is that data on obstetric morbidity were lacking for half of the investigated patients, excluding this APS criterion from many of the calculations. Finally, we did not have access to complete information about RF positivity, which made it impossible to properly estimate the possible degree of RF interference [36].

To conclude, we report modest agreement but similar association to previous thrombotic events comparing a new automated aPL method with standard assays in a large group of Swedish SLE patients. Interestingly, IgG antibodies were primarily associated with VTE and ICVD, while IHD was only associated with aPL of the IgM isotype. Our findings further suggest that antibody titres and the LA test are important when results from aPL testing are used for risk assessment of SLE patients. Overall, the LA test performed better than both of the investigated specific aPL assays with respect to association with thrombosis. Future evaluation of serological methods for APS diagnosis should be performed in prospective settings in order to shed further light on their clinical utility.

Rheumatology key messages

- We observed moderate agreement in SLE patients between a modern and a standard aPL assay.
- Two aPL assays performed equally well with respect to thrombotic manifestations in SLE patients.
- LA, antibody titres and antibody isotypes are important when using aPL results for risk assessment in SLE patients.

Acknowledgements

The authors would like to thank Sonja Möller, Susanne Pettersson, Eva Jemseby, Gullbritt Almgren, Julia Boström and Rezvan Kiani Dehkordi for blood sampling and laboratory assistance, and John Öhrvik for statistical advice. The authors are also grateful to Orgentec and Phadia for providing the test reagents.

Funding: This work was supported by Avtalet mellan Landstinget och Staten om samarbete om Läkarutbildning och Forskning (ALF) funding from Stockholm County Council, the Swedish Research Council, the Swedish Heart-Lung Foundation, the Swedish Rheumatism Foundation, the King Gustaf V 80th Birthday Fund, the Swedish Society of Medicine, the Åke Wiberg Foundation, the Alex and Eva Wallström Foundation, the Foundation in memory of Clas Groschinsky, Karolinska Institutet Foundations, the Medical Faculty at Lund University, Greta and Johan Kock's Foundation, Foundation of the National Board of Health and Welfare and Skåne University Hospital, Uppsala University Hospital Research and Development Fund, the Knut and Alice Wallenberg Foundation; the Ragnar Söderberg Foundation and COMBINE.

Disclosure statement: The authors have declared no conflicts of interest.

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