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ORIGINAL ARTICLE

Clinical accuracy for diagnosis of antiphospholipid syndrome in systemic lupus erythematosus: evaluation of 23 possible combinations of antiphospholipid antibody specificities

S. SCIASCIA,*† V. MURRU,* G. SANNA,*‡ D. ROCCATELLO,† M. A. KHAMASHTA*‡ and M. L. BERTOLACCINI*

**Lupus Research Unit, The Rayne Institute, Division of Women's Health, King's College London, London, UK; †Centro di Ricerche di Immunologia Clinica ed Immunopatologia e Documentazione su Malattie Rare (CMID), Università di Torino, Turin, Italy; and ‡Louise Coote Lupus Unit, Guy's and St Thomas' NHS Foundation Trust, St Thomas' Hospital, London, UK*

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Summary. *Objectives:* To evaluate the clinical accuracy of antiphospholipid antibody (aPL) specificities both individually and/or in combination, in a wide cohort of systemic lupus erythematosus (SLE) patients in an attempt to identify a panel of tests that may provide the best accuracy for diagnosing antiphospholipid syndrome (APS). *Patients and Methods:* This study included 230 patients (218 women, mean age 42.7 ± 11.9 years, mean disease duration 12.2 ± 8.7 years), all fulfilling the 1982 criteria for SLE. All patients were tested for lupus anticoagulant (LA), anti-cardiolipin (aCL), anti-$\beta_2$glycoprotein I (anti-β2GPI), solid phase anti-prothrombin (aPT), anti-phosphatidylserine/prothrombin (aPS/PT), and anti-phosphatidylethanolamine (aPE) antibodies. Sensitivity, specificity and predictive values were calculated. The diagnostic accuracy for each combination of tests was assessed by ROC and their area under the curve analysis as well as by the Youden's index (YI). *Results:* Testing for six aPL derived 23 possible combinations of results. Among them, LA + anti-β_2GPI + aPS/PT had the best diagnostic accuracy for APS as a whole and individually for both thrombosis and pregnancy loss (AUC 0.712, OR 3.73 [95% CI 1.82–5.38], $P = 0.0001$, YI = 0.32 and AUC 0.709, OR 3.75 [95% CI 2.13–6.62], $P = 0.0001$, YI = 0.37 and AUC 0.677, OR 4.82 [95% CI 2.17–10.72], $P = 0.0007$, YI = 0.38, respectively) and the best specificity when compared with all the other obtainable combination of tests. Triple positivity for LA + anti-β_2GPI + aPS/PT was more strongly associated with clinical events (thrombosis and/or PL) when compared with double or single positivity (OR 23.2 [95% CI 2.57–46.2] vs. OR 7.3 [95% CI 2.21–25.97], OR 5.7 [95% CI 2.12–17.01] or OR 3.11 [95% CI 1.56–7.8] for single positivity for LA, aPS/PT and anti-β_2GPI, respectively). *Conclusions:* Combining LA, anti-β_2GPI and aPS/PT improves the diagnostic power and helps in stratifying the risk for each patient, according to their aPL profile.

Keywords: aPL, Hughes syndrome, pregnancy loss, prothrombin, thrombosis.

Correspondence: Maria Laura Bertolaccini, Lupus Research Unit, The Rayne Institute, Division of Women's Health, King's College London, 4th Floor Lambeth Wing, St Thomas' Hospital, London SE1 7EH, UK.
Tel.: +44 2071883569; fax: +44 2076202658.
E-mail: maria.bertolaccini@kcl.ac.uk

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Introduction

The antiphospholipid syndrome (APS) is a thrombophilic disorder characterized by arterial and/or venous thrombosis and/or pregnancy loss, associated with the presence of a specific group of autoantibodies, the so-called antiphospholipid antibodies (aPL). In clinical practice, anticardiolipin (aCL) and anti-β_2 glycoprotein I (anti-β_2GPI) antibodies detected by an enzyme linked immunosorbent assay (ELISA) and the lupus anticoagulant (LA) detected by clotting assays are the most widely used tests for the detection of aPL. In addition, positivity for one or more of these three aPLs is a requirement to fulfill criteria for the classification of APS, along with at least one of the major clinical manifestations [1,2].

Several authors have suggested that testing for new aPL specificities may help to identify the syndrome in patients with thrombosis or pregnancy losses in whom APS is strongly suspected but conventional aPL are repeatedly negative [3], the so-called 'seronegative APS' [4]. In addition, several autoantibodies directed to proteins of the coagulation cascade (i.e. prothrombin) or their complex with phospholipids (i.e. phosphatidylserine–prothrombin) have been proposed to be relevant to APS [5], although the clinical utility and their diagnostic value remain undecided. Unfortunately, most of the studies are

based on testing for the distinct clinical significance of a particular antibody instead of establishing the potential additional value of an individual test or a combination of tests in the recognition of APS.

We designed this study to evaluate the clinical accuracy of known aPL specificities, both individually and in combination, in a wide cohort of patients with systemic lupus erythematosus (SLE) in an attempt to identify a panel of tests, or their combinations, that may provide the best accuracy for diagnosing APS.

Patients and methods

Patients

This study included 230 consecutive patients (218 women, mean age 42.7 ± 11.9 years, mean disease duration 12.2 ± 8.7 years), all fulfilling the 1982 criteria for SLE [6]. Of these, 61 patients fulfilled criteria for definite APS [1,2] and 55 were positive for aPL without fulfilling criteria.

Overall, 86 patients had a history of thrombosis (40 arterial, 26 venous and 20 both arterial and venous thrombosis). Out of 145 women who had ever been pregnant, 39 had a history of miscarriages (before the 10th week of gestation) and 36 a history of fetal death (after the 10th week of gestation). Demographic data are summarized in Table 1.

Ethical approval was obtained from the Guy's and St Thomas' Ethics Committee and all patients involved in this study gave their written consent.

Methods

All patient samples were obtained during a routine appointment. All aPL tests were performed on the same sample obtained on the day of the appointment after written consent was given.

LA determination Plasma samples were tested for the presence of LA according to the recommended criteria from the ISTH Subcommittee on Lupus Anticoagulant-Phospholipid-dependent antibodies [7], using the Automated Coagulation Laboratory (ACL) 300R (Instrumentation Laboratory, Milan, Italy). All samples were screened using the activated partial thromboplastin time (aPTT – IL test™ APTT-SP; Instrumentation Laboratory). Ratios higher than 1.10, which did not correct with the 50:50 mixture with normal plasma, were considered as suggestive of LA and subjected to dRVVT testing.

The dilute Russell viper venom time (dRVVT) coagulation test was performed using Diagen Russell's viper venom (Diagnostic Reagents Ltd, Oxon, UK) as described by Thiagarajan *et al.* [8] in all samples. Both screen and confirm steps were performed. Ratios higher than 1.10, which did not correct with the 50:50 mixture with normal plasma but decreased by 10% or more when using excess of phospholipids, were diagnostic of LA.

Other aPL testing aPL were tested for IgG and IgM isotypes. The aCL ELISA was performed according to the standardized technique [9]. Anti-β_2GPI was detected by ELISA as described previously [10], using purified human β_2GPI (Yamasa, Japan) coated on irradiated microtitre plates (Nunc Maxisorp, Denmark). Antibodies to prothrombin were tested by two methods, the aPT ELISA, using purified human prothrombin (Enzyme Research Laboratories, UK) coated on irradiated plates, as previously detailed [11], and the aPS/PT ELISA using purified human prothrombin/phosphatidylserine complex as antigen, as previously reported [12].

aPE were tested as described by Sanmarco *et al.* [13] using bovine brain phosphatidylethanolamine (Sigma-Aldrich, UK) [14].

The cut-off value for each aPL assay was determined by the 99th percentile of ≥ 100 healthy controls.

Table 1 Demographic characteristics of SLE

	SLE total n = 230 (%)	SLE/APS* n = 61 (%)	SLE/aPL (no APS)† n = 55 (%)	SLE only (no aPL) n = 114 (%)	P
Female (%)	218 (95)	56 (91)	52 (95)	106 (93)	NS
Mean age ± SD	42.7 ± 11.9	41.3 ± 10.8	43.8 ± 8.9	45.1 ± 10.1	NS
Mean disease duration ± SD	12.2 ± 8.7	10.9 ± 5.7	13.5 ± 8.3	12.9 ± 9.1	NS
Thrombosis‡	86 (37)	51 (84)	–	35 (31)	.039
Arterial thrombosis	60 (26)	33 (54)	–	27 (24)	.041
Venous thrombosis	46 (20)	28 (46)	–	18 (16)	.036
Pregnancy loss§	40 (27)	34 (75)	–	6 (10)	.031
Miscarriages (≥ 1)	39 (28)	25 (55)	3(7)	11 (19)	.037
Miscarriages (≥ 3)	9 (6)	4 (8)	–	5 (9)	.022
Fetal death	36 (25)	35 (73)	–	1 (2)	.001

SLE, systemic lupus erythematosus; APS, antiphospholipid syndrome; aPL, antiphospholipid antibodies, including aCL, LA and/or anti-β2GPI. *All patients fulfilled criteria for APS [2]. †aPL included patients who were positive for aPL but did not fulfill criteria (aPL but no clinical events attributable to APS). ‡Twenty patients from each group have both arterial and venous thrombosis. §Pregnancy loss was defined by APS criteria [2]. All pregnancy data percentages calculated over the total number of women who had ever been pregnant (n = 145 in total cohort, n = 45 in APS group, n = 42 in aPL and n = 58 in SLE).

Table 2 Prevalence of aPL in SLE

aPL*	SLE $n = 230$ (%)
LA	56 (25)
aCL IgG/IgM	126 (56)
aCL IgG	111 (49)
aCL IgM	46 (20)
Anti-β2GPI IgG/IgM	48 (21)
Anti-β2GPI IgG	38 (16)
Anti-β2GPI IgM	14 (6)
aPE IgG/IgM	92 (41)
aPE IgG	79 (35)
aPE IgM	24 (10)
aPT IgG/IgM	68 (30)
aPT IgG	57 (25)
aPT IgM	15 (6)
aPS-PT IgG/IgM	68 (30)
aPS-PT IgG	55 (24)
aPS-PT IgM	33 (14)

*Some patients were positive for more than one antibody and/or isotype. IgG/M, IgG and/or IgM; aPE, anti-phosphatidylethanolamine; aCL, anticardiolipin antibodies; anti-β2GPI, antibodies to β2 glycoprotein I; aPT, antibodies to prothrombin in solid phase; aPS-PT, antibodies to phosphatidylserine-prothrombin complex; LA, lupus anticoagulant.

Statistical analysis

Mann–Whitney *U*, Fisher's exact or chi-square tests were applied as appropriate. *P*-values < 0.05 were considered significant. Comparisons between groups were expressed as odds ratio with its 95% confidence interval (OR [95% CI]), where a lower limit > 1.0 was considered significant. Sensitivity, specificity and positive and negative predictive values (PPV and NPV) were calculated to compare the accuracy between the different combinations of tests. Areas under the receiver operating characteristic (ROC) curve (AUC) of different combinations of the six aPL tested were computed. The diagnostic accuracy for each combination of tests was also assessed based on Youden's J statistic (Youden's index). All statistical analyses were performed using SPSS 17.0 (IBM, Chicago, IL, USA).

Results

Prevalence of all aPL tested is shown in Table 2.

Patients were considered positive for aPL when any (at least one) of the six tested antibodies was positive. Overall, 61 patients were diagnosed as having APS and 55 patients showed positivity for LA, aCL and/or anti-β_2GPI in the absence of clinical events attributable to APS. When increasing the panel to six aPL, 177 patients (77%) were found to be positive for at least one of them.

Diagnostic performances for the combination of LA and aCL (Sapporo Laboratory Criteria) and LA + aCL + anti-β_2GPI (Sydney revised Criteria) were evaluated and compared with other possible combinations of tests (Tables 3 and 4).

The 'Sapporo combination' (LA + aCL) gave a sensitivity of 80%, a specificity of 44%, a PPV of 23% and an NPV of 91% for APS diagnosis (both thrombosis and/or pregnancy loss). The 'Sydney combination' showed a similar diagnostic performance (Table 4). Although the use of more than three

Table 3 Diagnostic accuracy evaluated by AUD through ROC

	APS			Thrombosis			Pregnancy loss		
Antibodies	AUC	OR [95% CI]	*P*	AUC	OR [95% CI]	*P*	AUC	OR [95% CI]	*P*
LA + aCL	0.612	3.22 [1.41–7.36]	0.0041	0.620	3.04 [1.67–5.52]	0.0002	0.613	1.70 [0.99–2.91]	0.0543
LA + aCL + anti-β2GPI	0.612	3.22 [1.41–7.36]	0.0041	0.620	3.04 [1.67–5.52]	0.0002	0.613	1.70 [0.99–2.91]	0.0543
LA + aCL + anti-β2GPI + aPS/PT	0.610	1.69 [0.89–2.96]	NS	0.599	3.05 [1.61–5.75]	0.0004	0.620	4.03 [1.50–10.79]	0.0033
LA + aCL + anti-β2GPI + aPT	0.594	1.74 [0.91–3.01]	NS	0.601	2.95 [1.56–5.58]	0.0007	0.584	2.46 [1.03–5.88]	0.0386
LA + aCL + anti-β2GPI + aPE	0.584	1.82 [0.84–4.62]	NS	0.599	2.82 [1.51–5.27]	0.0009	0.592	2.46 [1.03–5.88]	0.0386
LA + aCL + aPS/PT	0.610	1.70 [0.88–2.97]	NS	0.599	3.04 [1.67–5.52]	0.0002	0.620	4.03 [1.50–10.79]	0.0033
LA + aCL + aPE	0.584	1.57 [0.92–2.69]	NS	0.599	2.82 [1.51–5.27]	0.0009	0.592	2.46 [1.03–5.88]	0.0386
LA + aCL + aPT	0.594	1.70 [0.99–2.91]	NS	0.601	2.95 [1.56–5.58]	0.0007	0.584	2.46 [1.03–5.88]	0.0386
LA + anti-β2GPI + aPS/PT	0.712	3.73 [1.82–5.38]	0.0001	0.709	3.75 [2.13–6.62]	0.0001	0.677	4.82 [2.17–10.72]	0.0007
LA + anti-β2GPI + aPT	0.650	3.01 [1.75–5.19]	0.001	0.652	3.64 [2.07–6.42]	0.0001	0.646	3.46 [1.653–7.36]	0.0008
LA + anti-β2GPI + aPE	0.608	2.17 [1.28–3.67]	0.0038	0.607	2.51 [1.43–4.40]	0.0011	0.625	3.00 [1.38–6.50]	0.0041
aCL + anti-β2GPI + aPS/PT	0.614	1.76 [1.04–2.99]	0.0357	0.606	2.79 [1.54–5.08]	0.0006	0.612	3.13 [1.31–7.46]	0.0076
aCL + anti-β2GPI + aPT	0.591	1.68 [0.99–2.85]	0.052	0.600	2.62 [1.45–4.73]	0.0012	0.572	1.94 [0.89–4.23]	NS
aCL + anti-β2GPI + aPE	0.570	1.52 [0.90–2.58]	NS	0.587	2.33 [1.30–4.18]	0.0042	0.572	1.94 [0.89–4.23]	NS
Anti-β2GPI + aPS/PT + aPT	0.658	3.63 [2.07–6.36]	0.0001	0.643	3.35 [1.91–5.88]	0.0001	0.643	3.38 [1.59–7.19]	0.001
aPT + aPS/PT + aCL	0.590	1.70 [1.00–2.89]	0.0482	0.589	2.58 [1.38–4384]	0.0026	0.578	2.34 [0.98–5.60]	0.0516
aPT + aPS/PT + LAC	0.618	2.31 [1.36–3.93]	0.0018	0.609	2.58 [1.47–4.54]	0.0009	0.619	2.87 [1.32–6.22]	0.006
aPT + aPE + anti-β2GPI	0.581	2.00 [1.18–3.39]	0.0099	0.579	1.97 [1.14–3.42]	0.015	0.582	1.99 [0.96–4.10]	NS
aPT + aPE + aCL	0.555	1.47 [0.87–2.49]	NS	0.570	2.12 [1.15–3.90]	0.0151	0.553	1.17 [0.76–3.82]	NS
aPT + aPE + LAC	0.592	2.12 [1.25–3.59]	0.0051	0.593	2.41 [1.33–4.35]	0.0032	0.602	2.72 [1.18–6.23]	0.0153
Anti-β2GPI + aPE + aPS/PT	0.627	2.49 [1.46–4.24]	0.0007	0.609	2.58 [1.47–4.54]	0.0009	0.632	3.29 [1.48–7.32]	0.0024
aPE + aPS/PT + LAC	0.610	2.08 [1.23–3.52]	0.0062	0.612	2.65 [1.50–4.68]	0.0007	0.611	2.68 [1.24–5.81]	0.0104
aPE + aPS/PT + aCL	0.581	1.59 [0.94–2.81]	NS	0.582	2.44 [1.30–4.57]	0.0049	0.591	2.81 [1.12–7.07]	0.0234

Table 4 Sensitivity, specificity, PPV, NPV and Youden's Index* for APS diagnosis, thrombosis and pregnancy loss for each combination of aPL

	APS					Thrombosis					Pregnancy loss				
Antibodies	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	YI*	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	YI*	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	YI*
LA + aCL	80	44	23	91	0.24	76	47	46	77	0.23	68	44	52	60	0.12
LA + aCL + anti-β2GPI	80	44	23	91	0.24	76	47	46	77	0.23	68	44	52	60	0.12
LA + aCL + anti-β2GPI + aPS/PT	85	38	51	58	0.23	81	41	44	78	0.22	87	36	23	92	0.23
LA + aCL + anti-β2GPI + aPT	86	35	54	61	0.21	81	41	44	78	0.22	82	34	22	89	0.16
LA + aCL + anti-β2GPI + aPE	84	36	48	63	0.20	80	40	44	77	0.20	82	34	22	89	0.16
LA + aCL + aPS/PT	68	44	52	60	0.12	76	47	46	77	0.23	87	36	23	92	0.23
LA + aCL + aPE	67	43	52	59	0.10	80	40	44	77	0.20	82	34	22	89	0.16
LA + aCL + aPT	68	44	52	60	0.12	81	41	44	78	0.22	82	34	22	89	0.16
LA + anti-β2GPI + aPS/PT	57	75	63	64	0.32	68	69	52	77	0.37	77	61	29	91	0.38
LA + anti-β2GPI + aPT	56	70	63	63	0.26	68	62	52	76	0.30	72	56	27	90	0.28
LA + anti-β2GPI + aPE	59	60	57	61	0.19	68	53	46	74	0.21	75	50	25	89	0.25
aCL + anti-β2GPI + aPS/PT	64	49	53	60	0.13	76	45	45	76	0.21	82	39	23	91	0.21
aCL + anti-β2GPI + aPT	62	50	53	59	0.12	78	45	45	75	0.23	75	39	21	87	0.14
aCL + anti-β2GPI + aPE	62	47	52	58	0.09	74	44	44	74	0.18	75	39	21	87	0.14
anti-β2GPI + aPS/PT + aPT	53	70	67	64	0.23	67	61	51	76	0.28	72	56	27	90	0.28
aPT + aPS/PT + aCL	64	48	53	59	0.12	80	38	43	76	0.18	82	33	21	89	0.15
aPT + aPS/PT + LAC	55	65	59	61	0.20	69	52	46	74	0.21	75	48	24	89	0.23
aPT + aPE + anti-β2GPI	52	64	57	59	0.16	65	51	44	71	0.16	67	48	22	87	0.15
aPT + aPE + aCL	62	46	51	57	0.08	77	37	42	73	0.14	77	33	20	86	0.10
aPT + aPE + LAC	61	56	56	61	0.17	78	43	44	75	0.21	80	40	23	90	0.20
anti-β2GPI + aPE + aPS/PT	56	65	60	62	0.21	69	52	46	74	0.21	77	48	25	90	0.25
aPE + aPS/PT + LAC	56	61	57	60	0.17	70	52	46	75	0.22	75	47	24	89	0.22
aPE + aPS/PT + aCL	64	64	52	58	0.28	80	37	43	76	0.17	85	33	22	90	0.18

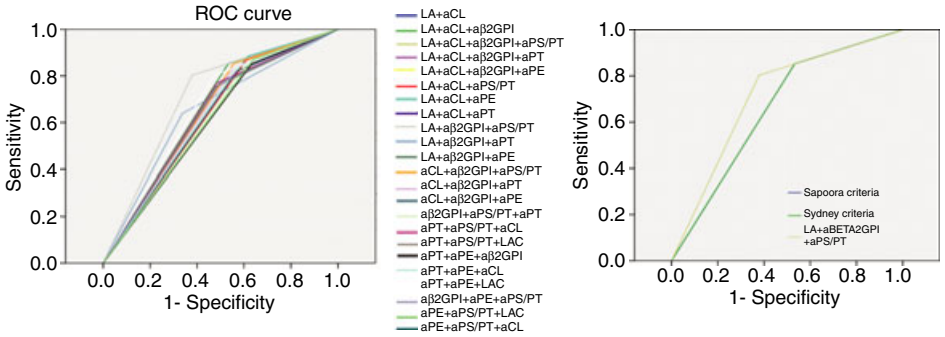

Fig. 1. (A) Receiver operating characteristic (ROC) curves of the 23 different aPL combinations. Sensitivity and specificity were calculated according to the presence of a history of thrombosis and/or PL. (B) ROC curves for Sapporo and Sydney criteria for APS in comparison with the combination including LA, aβ_2GPI and aPS/PT. AUC are 0.612, 0.612 and 0.712, respectively.

tests increased the overall sensitivity for APS diagnosis to over 80%, it deeply impacted on the specificity, which dropped to under 40% for all the combinations (LA + aCL + anti-β_2GPI + aPS/PT = 38%; LA + aCL + anti-β_2GPI + aPT = 35%; LA + aCL + anti-β_2GPI + aPE = 36%). Among the different combinations of three available tests, LA + anti-β_2GPI + aPS/PT had the best specificity (Table 4).

When analyzing APS diagnosis, the AUC for Sydney criteria was 0.612 (Fig. 1). The higher values of the AUC were achieved by the combination of LA + anti-β_2GPI + aPT/PS (AUC 0.712, OR 3.89 [95% CI 1.96–5.38], P = 0.0001) (Fig. 1).

The Sapporo combination (aCL + LA) gave a sensitivity of 76%, a specificity of 47%, a PPV of 46% and an NPV of 77% for thrombosis. The Sydney combination had an equivalent diagnostic performance for thrombosis (Table 4). As observed for APS diagnosis, the use of more than three tests showed an increase in the overall sensitivity for thrombosis to over 80%, but reduced the specificity to around 40% (LA + aCL + anti-β_2GPI + aPS/PT = 41%; LA + aCL + anti-β_2GPI + aPT = 41%; LA + aCL + anti-β_2GPI + aPE = 40%). Among the different combinations of three available tests, LA + anti-β_2GPI + aPS/PT was confirmed as having the best specificity for thrombosis (69%) when compared with an average 47% for all the other combinations of three tests (range 37%–62%).

For pregnancy loss, only patients who fulfilled criteria [2] (i.e. ≥ 3 miscarriages and/or ≥ 1 fetal death) were analyzed. The Sapporo combination gave a sensitivity of 68%, a specificity of 44%, a PPV of 52% and an NPV of 60%. The Sydney combination had identical diagnostic performance (Table 4).

As noted before, the use of more than three tests increased the overall sensitivity for pregnancy loss to over 80%, but decreased the specificity to 36% for LA + aCL + anti-β_2GPI + aPS/PT and 34% for LA + aCL + anti-β_2GPI + aPT and LA + aCL + anti-β_2GPI + aPE. Among the different combinations, LA + anti-β_2GPI + aPS/PT was confirmed as having the best specificity for pregnancy loss (61%) when compared with an average 43% for all the other combinations (range 33%–56%).

In addition, this combination of LA + anti-β_2GPI + aPS/PT had a better diagnostic performance for pregnancy loss than the Sapporo and Sydney criteria combinations, both in sensitivity and specificity (Table 2).

The AUC data were confirmed by Youdon's Index (YI), showing best diagnostic performances for the LA + anti-β_2GPI + aPS/PT combination for APS, thrombosis and PL (AUC 0.712, OR 3.73 [95% CI 1.82–5.38], P = 0.0001, YI = 0.32; AUC 0.709, OR 3.75 [95% CI 2.13–6.62], P = 0.0001, YI = 0.37 and AUC 0.677, OR 4.82 [95% CI 2.17–10.72], P = 0.0007, YI = 0.38, respectively).

In addition, we performed a further analysis to investigate the clinical risk in the presence of single, dual or multiple positivity including LA + anti-β_2GPI + aPS/PT. As shown in Fig. 2, concomitant triple positivity for LA, aβ_2GPI and aPS/PT was more strongly associated with clinical events (thrombosis and/or pregnancy loss) when compared with double or single positivity (OR 23.2 [95% CI 2.57–46.17] vs. OR 7.32 [95% CI 2.21–25.97], OR 5.67 [95% CI 2.12–17.01], OR 3.11 [95% CI 1.56–7.77] for single positivity for LA, or aPS/PT or aβ_2GPI, respectively) (Fig. 2).

Discussion

Vascular thrombosis and pregnancy morbidity were described as the main clinical features of APS in the early 1980s [15], deep venous thrombosis and pregnancy losses being the most

		aβ2-GPI+ve	aβ2-GPI–ve
LA+VE	aPS/PT+ve	23.2 [2.57–46.17]	10.47 [2.21–26.97]
	aPS/PT–ve	13.78 [2.04–16.33]	7.32 [2.67–25.97]
LA–VE	aPS/PT+ve	9.13 [2.17–15.62]	5.67 [2.12–17.01]
	aPS/PT–ve	3.11 [1.56–7.77]	

Fig. 2. Odd ratios for thrombosis are estimated according to aPL profile, showing that each further positivity increases the risk of event. Multiple aPL positivity, particularly the triple association of LA and aβ2GPI and aPS/PT further increases the risk of thrombosis.

common. As both these events are relatively common in the general population and in subjects with autoimmune diseases, correctly classifying patients with APS can be a complex task. In addition, patients who experience thrombosis or recurrent miscarriages are classified as having APS based exclusively on the presence of routinely tested aPL (i.e. aCL, LA and in some laboratories but not all, anti-β_2GPI). Therefore, laboratory testing for aPL has an extraordinarily critical role in the clinical setting.

In clinical practice, aCL and anti-β2GPI antibodies detected by ELISA and LA detected by clotting assays have been the most established tests for diagnosis of APS [16]. However, the family of aPL is continuously expanding to include a heterogeneous group of autoantibodies whose specificity is directed against phospholipid binding proteins or their complex with phospholipids. In addition, a wide variability in strength of association between routine and newly tested aPL and the clinical manifestations of APS have also been reported. In the search for better markers for APS, most of the attention has been focused on describing new specificities for aPL and very little on the evaluation of the potential best combination of the already available tests.

Recent studies have shown that the risk of thrombotic events increases with the number of positive tests in APS patients [17–19] and aPL carriers [20]. These studies focused on the routinely tested aPL (i.e. aCL, anti-β2GPI and LA). Pengo *et al*. [21] suggested that positivity in a single test among LA, aCL and aβ_2GPI would call the diagnosis of APS into question and, conversely, suggested that triple positivity is strongly associated with thrombosis and pregnancy loss [20,21].

In this study, we retrospectively analyzed a large series of SLE patients, and assessed the potential clinical usefulness of combining routinely tested aPL with new aPL specificities in an attempt to find a profile that will identify patients at higher risk of APS. Among the 23 possible combinations of the six aPL tested, LA + anti-β_2GPI + aPS/PT had the best diagnostic accuracy for APS as a whole, and for both thrombosis and pregnancy loss (PL). When comparing it to the combination suggested by the current criteria and previous studies [18] and all the other tested combinations, positivity for LA + anti-β_2GPI + aPS/PT had the best diagnostic performance in terms of specificity and PPV in our SLE cohort. In this case, the increased specificity was due to anti-β2GPI being a more specific marker than aCL [22].

Besides, some of the proposed combinations, namely the combination of four tests (Sydney revised laboratory criteria plus aPE and/or aPT and/or aPT/PS, respectively) presented AUC under the value 0.6, suggesting that the sole increase in sensitivity given by the use of more tests does not improve the diagnostic performance.

In addition, we found that simultaneous positivity, double or triple, was detected more frequently in patients with thrombosis. Interestingly, also in the combination LA + anti-β_2GPI + aPS/PT, each further aPL positivity detection increased the risk of thrombosis, with OR ranging from three to seven for the single positivity for anti-β_2GPI and aPS/PT, respectively, to 23 for the triple positivity (Fig. 2). We found that triple positivity for LA + anti-β_2GPI + aPS/PT was the strongest risk factor for thrombosis and/or pregnancy loss (OR 23.2) even when comparing it with data reported in the current literature about triple positivity for LA + aCL + anti-β_2GPI (OR 14.9) [18]. These findings are in line with data recently reported by Otomo *et al*. showing that the inclusion of aPS/PT in the battery of aPL tests allowed a better quantification of the thrombotic risk [23].

It is also true that our model has some limitations as we used dichotomized variables. This strategy simplified the comparison of the different combinations of tests. Nevertheless, the use of titer for each aPL test as continuous variable did not provide more refined information and confirmed the results obtained by using dichotomized variables (data not shown).

Thus, our data confirm that triple positivity for aPL identifies patients at high risk of thrombotic events and obstetric complications. The concomitant triple positivity for LA + anti-β_2GPI + aPS/PT is not only strongly associated with thrombosis and pregnancy loss, but shows a higher diagnostic accuracy than that of aCL + LA + anti-β2GPI at least in this cohort of patients with SLE.

In summary, combining LA + anti-β_2GPI + aPS/PT not only improves the diagnostic power but seemed to be helpful in stratifying the risk of an event, according to the aPL profile.

Our study may lead to a differentiated aPL testing approach (aPL screening and aPL confirm), to be confirmed in larger prospective studies.

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Disclosure of conflict of interests

The authors state that they have no conflict of interest.

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