

PAPER

Diagnosis of antiphospholipid syndrome in routine clinical practice

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The updated international consensus criteria for definite antiphospholipid syndrome (APS) are useful for scientific clinical studies. However, there remains a need for diagnostic criteria for routine clinical use. We audited the results of routine antiphospholipid antibodies (aPLs) in a cohort of 193 consecutive patients with aPL positivity-based testing for lupus anticoagulant (LA), IgG and IgM anticardiolipin (aCL) and anti- β_2 glycoprotein-1 antibodies (a β_2 GPI). Medium/high-titre aCL/a β_2 GPI was defined as >99th percentile. Low-titre aCL/a β_2 GPI positivity (>95th < 99th percentile) was considered positive for obstetric but not for thrombotic APS. One hundred of the 145 patients fulfilled both clinical and laboratory criteria for definite APS. Twenty-six women with purely obstetric APS had persistent low-titre aCL and/or a β_2 GPI. With the inclusion of these patients, 126 of the 145 patients were considered to have APS. Sixty-seven out of 126 patients were LA-negative, of whom 12 had aCL only, 37 had a β_2 GPI only and 18 positive were for both. The omission of aCL or a β_2 GPI testing from investigation of APS would have led to a failure to diagnose APS in 9.5% and 29.4% of patients, respectively. Our data suggest that LA, aCL and a β_2 GPI testing are all required for the accurate diagnosis of APS and that low-titre antibodies should be included in the diagnosis of obstetric APS. *Lupus* (2013) 22, 18–25.

Introduction

The antiphospholipid syndrome (APS) is characterized by thrombotic and/or pregnancy morbidity associated with the presence of persistent antiphospholipid antibodies (aPLs).¹ There are many other clinical manifestations associated with persistent aPL (including immune thrombocytopenia, livedo reticularis, migraine, valvular heart disease and cognitive dysfunction), and, while these conditions are not considered diagnostic for APS, they are frequently encountered and require clinical attention.

The updated international consensus (Sydney) classification (ICS) criteria for definite antiphospholipid syndrome¹ require the presence of a lupus anticoagulant (LA) and/or IgG or IgM anticardiolipin antibodies (aCL) present in medium or high titre (i.e. >40 GPL or MPL or >99th percentile), and/or anti- β_2 glycoprotein-1 (a β_2 GPI) (IgG and/or IgM) >99th percentile. These aPL should be persistent, defined as being present on two or more consecutive occasions at least 12 weeks

apart. The international consensus criteria were originally designed for scientific clinical studies and were never intended for diagnostic use. Consequently, there remains a need for firm diagnostic criteria for routine clinical use, which may differ from these.

The criteria for the laboratory diagnosis of APS remain controversial. It has been proposed by some that the Sydney laboratory criteria should be modified such that testing for a β_2 GPI should be limited to measurements of IgG a β_2 GPI only and testing for aCL should be omitted.² The basis for this is that in a systematic review, LA showed the highest strength of association with thrombotic complications^{3,4} and IgG but not IgM a β_2 GPI was associated with thrombosis. In addition, Opatrny et al. reported in a meta-analysis that LA was also most strongly associated with late (>13 and <24 weeks) recurrent fetal loss.⁴ Galli et al.³ also drew attention to the need to produce guidelines, which were subsequently published,⁵ attempting to standardize more clearly the criteria for the detection of LA.

Others have argued that it is premature to consider reducing the number of assays used in the diagnosis of APS. The systematic review by Galli et al.³ referred to above also suggested that medium- or high-titre IgG aCL may represent a

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possible risk factor for thrombosis. We and others have previously reported that omission of aCL testing from the clinical investigation of APS could lead to a failure to diagnose the syndrome in a proportion of patients,^{6–8} and, in a multicentre prospective European women cohort, isolated aCL and/or a β_2 GPI positivity was found in a proportion of women with obstetric APS.⁷ The cut-off for serological positivity is also contentious. It has been reported that women with obstetric APS (without systemic thromboembolism) have lower aCL antibody titres than patients with a thrombotic history.⁹ Data from a retrospective cohort study¹⁰ and also in the prospective European cohort⁷ suggest that low-titre aCL, defined as those between the 95th and 99th percentiles rather than the 99th percentile as suggested in the ICS criteria, are of clinical significance for women with purely obstetric APS.

Wahl et al. suggested that modifications of the serological criteria for the diagnosis of APS should in the future be based on new data and on appropriate systematic reviews.⁸ The proposed entity of seronegative APS, where patients have characteristic clinical manifestations of APS but lack conventional serological markers, has also been given consideration in classification criteria for APS.¹¹ We report on serological criteria in a cohort of patients diagnosed to have APS, based on a comprehensive methodological approach which included testing for LA as well as IgG and IgM aCL and a β_2 GPI.

Methods

Patients and samples

We audited data on routine aPL testing retrospectively from a cohort of 193 consecutive patients attending the thrombosis and haemostasis, recurrent miscarriage or high-risk antenatal clinics at UCLH, who had persistent aPL positivity based on testing for LA, IgG and IgM aCL and a β_2 GPI on two or more consecutive occasions at least 12 weeks apart. Case ascertainment was based on review of the clinic letters of all patients attending the clinics specified above. These clinic letters were saved prospectively in a dedicated area on the hospital electronic records system so that they were all immediately retrievable.

In patients with thrombotic APS, *high-risk* patients have been recognized in the literature to include those who experience recurrent venous events or arterial thrombosis.^{12,13} However, there are no agreed published definitions for the categorization of the clinical severity of thrombotic, or

obstetric, APS. In this audit, *high-risk APS* was defined as the presence of any of the following clinical scenarios: recurrent objectively diagnosed thrombotic events; thromboses in both venous and arterial sites; both early and late pregnancy morbidity as defined in the Sydney clinical criteria for APS¹; both pregnancy morbidity and thrombotic events; and/or thrombosis or pregnancy morbidity whilst receiving anticoagulant therapy. The remainder were categorized as *lower-risk*.

Venous blood for LA was collected into 5 ml tubes containing one-tenth volume 0.105 M trisodium citrate (e.g. Vacutainer[®], Becton Dickinson, Plymouth, UK) using 19 or 21 gauge needles and minimal stasis. For LA, platelet-poor plasma was prepared by double centrifugation at room temperature at 2000 *g* for 15 minutes, and frozen in aliquots at -80°C until assayed. a β_2 GPI antibodies and aCL were performed on serum.

Antiphospholipid antibody assays

All patients in the study cohort had persistent aPL—that is, aPL were present on two or more consecutive occasions at least 12 weeks apart.

aCL assays were performed using an in-house assay employing 10% fetal calf serum as a blocking agent¹⁴ based on the work of Loizou et al.¹⁵ and standardized using the polyclonal ‘Harris’ standards.^{15,16} The Sydney laboratory criteria state that medium- or high-titre aCL are those above the 99th percentile or 40 GPLU/MPLU. In this study, aCL positivity was defined as: medium/high titre (99th percentile) >20 GPLU/MPLU; low-titre (95–99th percentile) IgG > 5 GPLU/MPLU. For patients with thrombotic APS, the 99th percentile cut-off was used to define aPL positivity. However, a number of studies have suggested the 99th percentile is too insensitive for clinical use for women with fulfilling Sydney clinical criteria for obstetric APS (and no systemic thrombotic manifestations)^{7,9,10,17} so the 95th percentile value was used to define in these patients.

β_2 GPI were measured using a commercial kit (Axis; Shield Diagnostics, Dundee, UK), based on a method developed in our department.¹⁸ a β_2 GPI positivity was defined as: low-titre (95–99th percentile) IgG > 3.5 units, IgM > 3.0 units; medium/high-titre (99th percentile) >15 units.

Reference ranges for aCL and a β_2 GPI were obtained from 240 normal healthy volunteers LA testing and derivation of the local reference range were performed according to ISTH¹⁹ and BCSH guidelines²⁰ current at that time, prior to publication of the updated ISTH criteria.⁵ Briefly, samples were

screened using the activated partial thromboplastin time (Pathromtin SL; Dade Behring, Marburg, Germany) and appropriate mixing studies. LA activity was confirmed using a dilute Russell's viper venom time (DRVVT) employing a platelet neutralization procedure (Pathway Diagnostics, Dorking, UK). Patients receiving warfarin were tested using the Taipan venom time as previously described.^{20,21} All patients had at least one DRVVT performed whilst not receiving warfarin.

Statistical analysis

Non-parametric statistical methods were used throughout. The Mann–Whitney *U* test was used to test for statistically significant differences between medians. Linear-by-linear association was assessed using the Mantel–Haenszel chi-squared test. Statistical significance was defined as $p < 0.05$. Statistical analyses were performed using SPSS 16.0 (SPSS Inc. Chicago, IL).

Results

Figure 1 shows a flowchart of patient selection according to Sydney clinical criteria for APS, with clinical diagnoses summarized here. One hundred

and forty-five of the cohort of 193 patients had the following clinical manifestations which fulfilled the Sydney clinical criteria 1: thrombosis (total 91; arterial 33; venous 48; both arterial and venous 10); early and/or late pregnancy morbidity as defined in the Sydney clinical criteria for APS (54), with 14 of these 54 exhibiting both thrombotic and obstetric manifestations. Nineteen of the 145 had underlying autoimmune disease (including 11 with systemic lupus erythematosus (SLE), two of whom also had ITP, and a further two with ITP). The remaining 48 of the 193 patients, who did not meet the Sydney clinical criteria, had the following diagnoses: immune thrombocytopenia (6), dystonia (4), migraine (12), multiple sclerosis (3), dementia (1), leprosy (1) or SLE (1). Eight were referred with a history of obstetric morbidity which did not meet the Sydney criteria and 12 were asymptomatic. These 48 patients will not be discussed further.

The distribution of aPL positivity is summarized in Figure 2 and by clinical diagnoses in Table 1. Of the 145 patients who fulfilled the Sydney clinical criteria for APS, 100 also fulfilled the Sydney laboratory criteria; that is, if aCL and/or aβ₂GPI were present their levels were greater than the 99th percentile. A further 26 women who fulfilled clinical criteria for purely obstetric APS (and without a

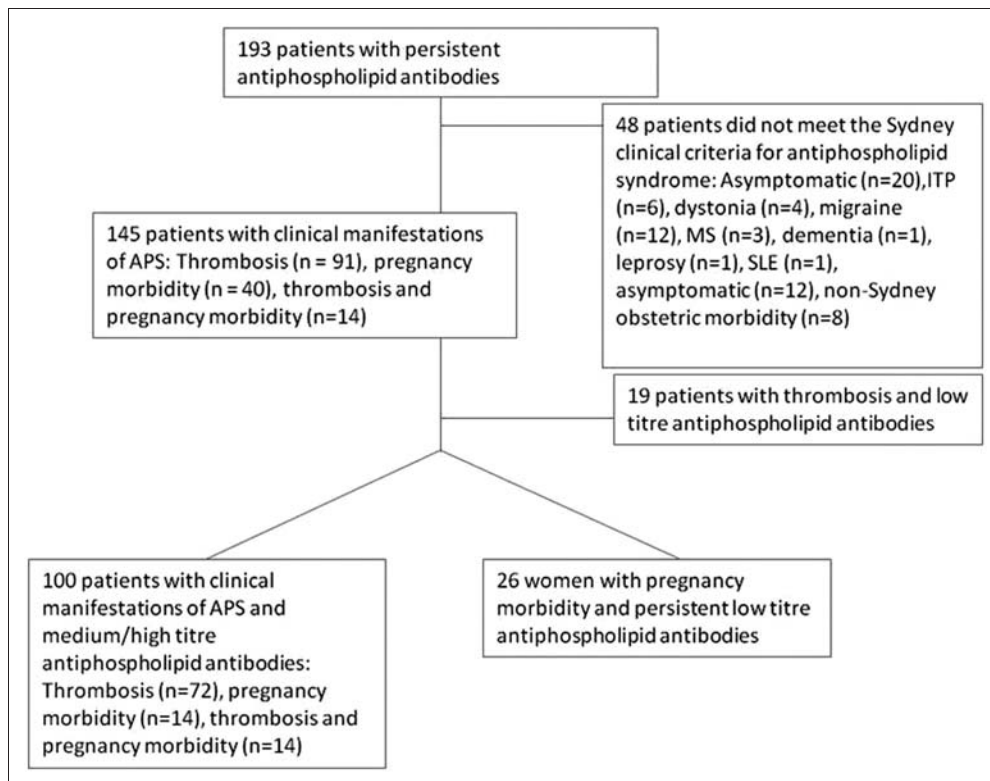


Figure 1 Flowchart showing patient selection according to Sydney clinical criteria for APS.

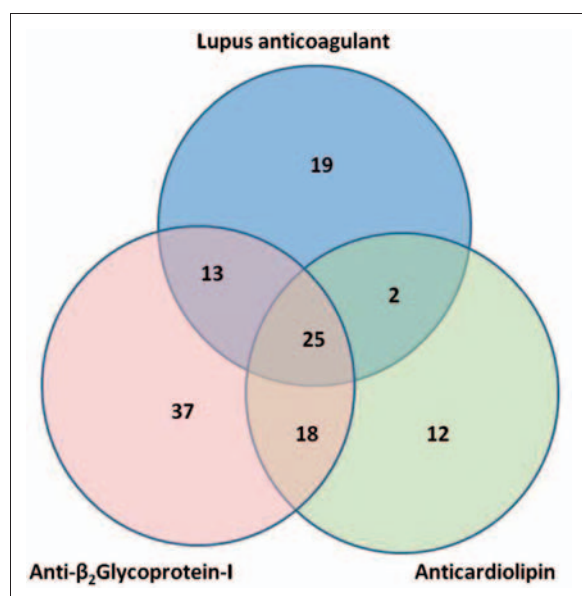


Figure 2 Distribution of antiphospholipid positivity in 126 patients with antiphospholipid syndrome as detailed in Figure 1.

history of thrombosis) had persistent low-titre aCL and/or aβ₂GPI; that is, above the 95th percentile but below the 99th percentile, in the absence of LA. Thus a total of 126/145 patients were considered to have APS according to our local criteria. Sixty-seven of these 126 patients were LA-negative, of whom 12 had aCL positivity only, 37 had aβ₂GPI positivity only, and 18 were positive for both aCL and aβ₂GPI. Consequently, omission of aCL or aβ₂GPI from the laboratory investigation of APS would have resulted in the failure to diagnose APS in 9.5% and 29.4% of patients, respectively. Twenty-two of these 67 patients had *high-risk APS* as defined in the Methods section above, of whom 11.5% had single positivity for aβ₂GPI or aCL. IgM aCL and/or aβ₂GPI antibodies (1 aCL, 25 aβ₂GPI, 3 aCL and aβ₂GPI), alone or in association with IgG antibodies, were found in 53.7% (29/54) of women who fulfilled Sydney clinical criteria for purely obstetric APS.

IgG aCL and IgM aCL levels (Figure 3) and IgM aβ₂GPI levels (Figure 4) were significantly higher in patients with a history of thrombosis than in women with a history of purely obstetric APS ($p < 0.05$). Similarly the rate of LA positivity was also significantly higher in patients with a history of thrombosis compared with those with obstetric APS alone (50.5% v 15%; $p = 0.0002$).

High-risk APS, as defined in the Methods section, was observed in 60 of the 145 (41%) patients fulfilling the Sydney clinical criteria for thrombosis

or obstetric APS. The number of positive tests (as defined by the Sydney laboratory criteria) was significantly associated with clinical severity in patients who fulfilled both clinical and laboratory Sydney criteria: 42.2% with one positive test, 41.2% with two positive tests; and 64.7% with three positive tests ($p = 0.026$). In contrast, only 30.0% of patients with low-titre aPL alone had a clinical history consistent with *high-risk APS* as defined in the Methods section.

With regard to the association between positivity for a single aPL test and severity of APS, only the incidence of LA (50.0% v 31.8%, $p < 0.05$) and median titre of IgM aCL (Figure 5) were associated with *high-risk APS* in this cohort. Twenty-five out of 54 (46.3%) women with a history of pregnancy morbidity had clinical histories consistent with *high-risk APS* (as defined in the Methods section) compared to 7/26 (26.9%) of the same group who had low-titre APL.

Discussion

We report the clinical and laboratory findings of a cohort of 193 patients who had persistently positive tests for aPL. One hundred of these 193 patients had APS as defined by Sydney clinical as well as laboratory criteria¹; and 126 when women with purely obstetric APS (without a history of thrombosis) associated with low-titre aCL and/or aβ₂GPI were also included as suggested in a number of other studies^{7,9,10,17} although not included in the Sydney laboratory criteria for APS.1.

Our data indicate that omission of testing for aCL or aβ₂GPI from the clinical investigation of APS would have led to a failure to diagnose the syndrome in 9.5% and 29.4% of patients respectively. Although the term *high-risk* in relation to patients with thrombotic APS now appears in the literature,^{12,13,22} there are no agreed published definitions for this term in thrombotic or obstetric APS. To assess the clinical relevance of persistent aPL in our cohort, as it is possible that these persistent aPL could have occurred by chance, we categorized patients in our cohort as having *high-risk* or *lower-risk APS* as detailed in the Methods section and correlated aPL results with clinical risk. We found, in agreement with previous reports, that LA as well as triple positivity (that is, aCL,²³ aβ₂GPI and LA) were associated with *high-risk APS*.^{3,22,24,25} Furthermore, 11.5% of patients with single positivity for aβ₂GPI or aCL (including IgM aCL) had *high-risk APS*, highlighting the

Table 1 The distribution of antiphospholipid positivity by clinical diagnoses

	Pregnancy morbidity (n = 40)		Thrombosis (n = 105 ^a)	
	>99 th percentile	>95 th < 99 th percentile	>99 th percentile	>95 th < 99 th percentile
IgG aβ ₂ GPI	4 (10.0%)	6 (15.0%)	30 (28.6%)	40 (38.1%)
IgM aβ ₂ GPI	6 (15.0%)	23 (57.5%)	25 (23.8%)	59 (56.2%)
IgG aCL	4 (10.0%)	13 (32.5%)	23 (21.9%)	45 (42.9%)
IgM aCL	0 (0.0%)	4 (10.0%)	10 (9.5%)	21 (20.0%)
LA positivity	6 (15.0%) ^b		53 (50.5%) ^b	

^aIncludes 14 women with both thrombosis and pregnancy morbidity.

The number (percentage) of positive tests and median antibody levels (>99th or >95th < 99th percentile) are given for each clinical group.

^bChi-squared: patients with pregnancy morbidity versus those with thrombosis $p=0.0002$.

aβ₂GPI: anti-beta₂ glycoprotein-1 antibodies; aCL: anticardiolipin antibodies; LA: lupus anticoagulant.

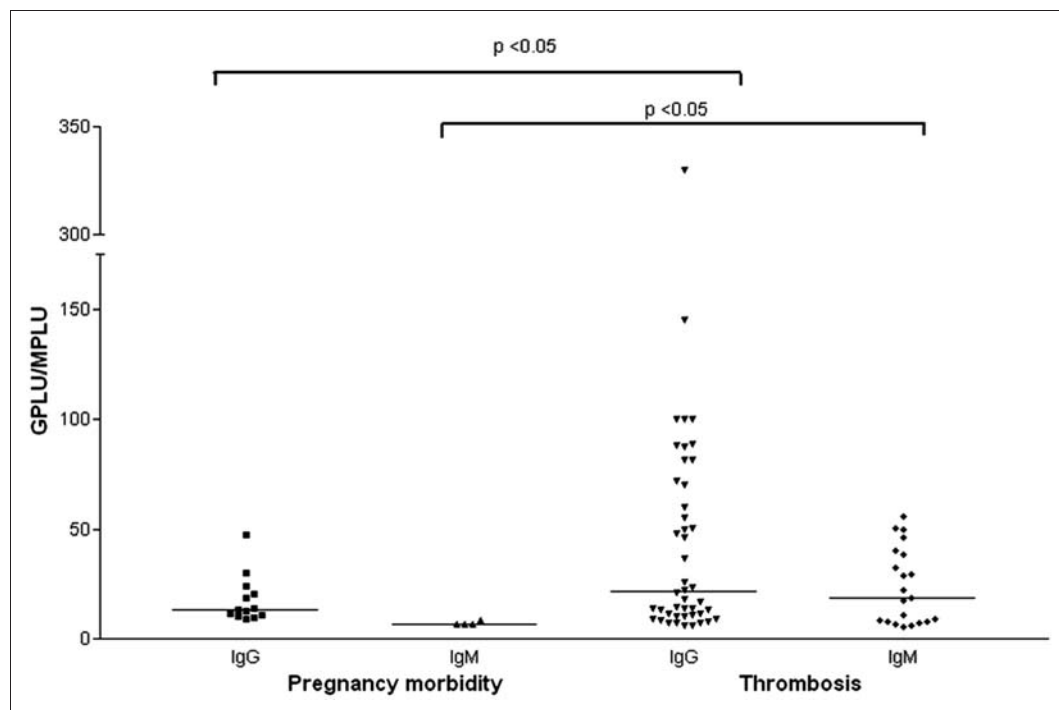


Figure 3 Anticardiolipin (aCL) results by clinical history. Pregnancy morbidity (n = 40), Thrombosis (n = 105, including 14 with both thrombosis and pregnancy morbidity). All aCL values above the 95th percentile are shown. Horizontal bars represent the median titres.

importance of inclusion of these tests in the diagnosis of APS, as previously reported.^{7,26–28}

Over 50% of women with clinical features of obstetric APS, but no thrombosis, had low-titre aCL and/or aβ₂GPI in the absence of LA. Approximately 27% of these patients had clinical features of *high-risk APS*. The association of low-titre aCL or aβ₂GPI in women with clinical features consistent with Sydney clinical criteria for obstetric APS could have arisen by chance; however, our findings concur with those of Ruffati *et al.*⁹ who reported that the rate of aCL values between the

99th percentile and 40 GPL units was significantly higher ($p < 0.0001$) in patients with pregnancy morbidity (73.7%) as compared to those with vascular thrombosis (16.9%) and those with both conditions (16.7%), and concluded that the 99th percentile cut-off level appears more sensitive than the >40 GPL value for the diagnosis of APS in women with persistent aCL positivity alone associated with obstetric APS. Low-titre aCL or aβ₂GPI, in some cases occurring as an isolated phenomenon, also appeared to be clinically significant in an analysis of laboratory findings in a

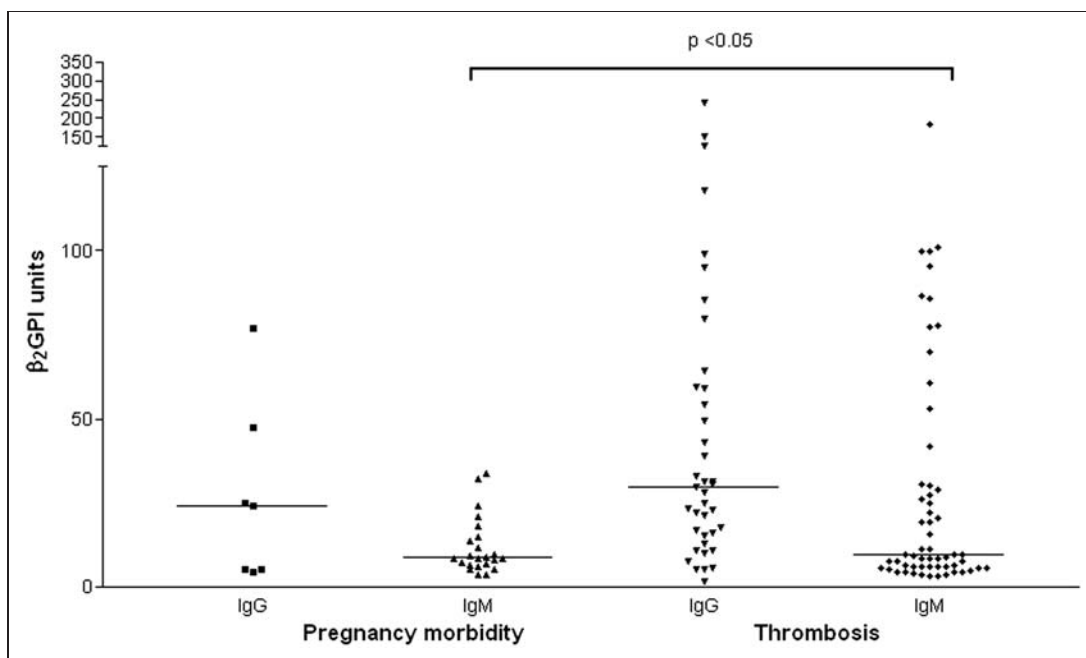


Figure 4 anti- β_2 glycoprotein-1 (β_2 GPI) results by clinical history. Pregnancy morbidity ($n = 40$), thrombosis ($n = 105$, including 14 with both thrombosis and pregnancy morbidity). All β_2 GPI values above the 95th percentile are shown. Horizontal bars represent the median titres.

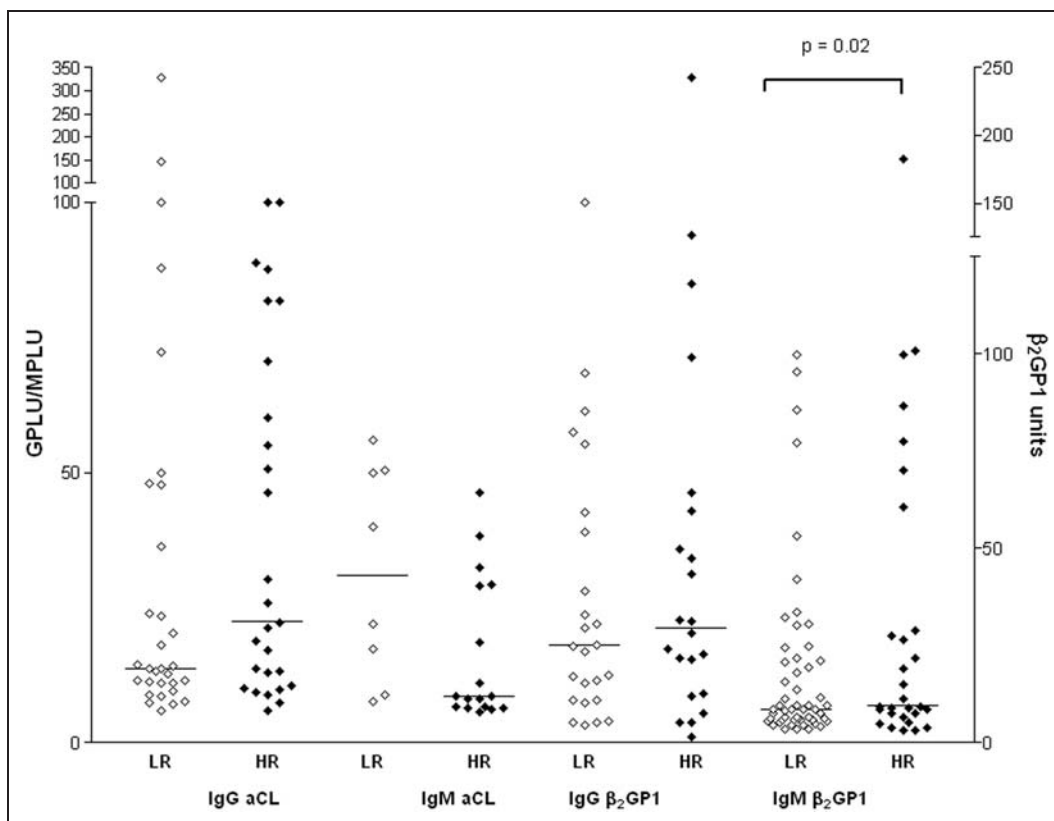


Figure 5 Antiphospholipid antibody results by clinical risk: *low-risk antiphospholipid syndrome (APS)* $n = 85$, *high-risk APS* $n = 60$. Horizontal bars represent the median titres.

prospective European cohort of women with obstetric APS.⁷ Furthermore, in clinical studies, persistent low-titre aCL were associated with a >90% fetal loss rate in untreated pregnancies of women with recurrent miscarriage and aPL, and with significantly improved pregnancy outcome following treatment with low-dose aspirin or heparin and aspirin.^{10,29–31} Our data also support the inclusion of low-titre IgG and IgM aCL and a β_2 GPI antibodies in the diagnosis of APS in women with a history of clinical obstetric APS, where accurate diagnosis has therapeutic implications during pregnancy with potentially far-reaching adverse clinical consequences in an infant who may suffer long-term physical disability and mental impairment as a result of placental-mediated obstetric morbidity such as intrauterine growth restriction, early onset pre-eclampsia or placental insufficiency/abruption.

The pattern of aPL positivity in our cohort differs from that in some published cohort studies. There may be several reasons for this. First, the aCL ELISA used in this study does not contain additional purified β_2 GPI and uses fetal calf serum as a blocking agent. Despite efforts to standardize solid phase assays for aCL, agreement between laboratories remains poor.^{32,33} The source of cardiolipin and technique used to coat the microtitre plates is known to affect results.³³ Some methods employ fetal calf serum as a blocking agent, while others use cardiolipin saturated with human β_2 glycoprotein-1 as the solid phase antigen. The situation for a β_2 GPI antibody tests is marginally better, with some agreement between most assays.^{34,35} However, different β_2 GPI purification methods are known to give rise to inter-assay variation.^{35,36} It should be noted that when human β_2 GPI is added to aCL assays there is understandably a high degree of agreement between aCL and a β_2 GPI assays³⁷ and this may have led to the suggestion that a β_2 GPI are not present in patients who are negative for aCL. Conversely, some have argued that aCL assays detect clinically significant antibodies which are not detected by a β_2 GPI assays.^{4,6,7} Secondly, a significant proportion of our cohort (37%) had purely obstetric APS whereas many published studies have included only patients with a history of thrombosis. Much of the published literature on aPL phenotypes in APS relates to patients with SLE-associated APS, whereas in the patients in our cohort only a minority (19/145) had SLE, as the study cohort comprised patients who had predominantly presented to haematology rather than rheumatology clinics.

In selecting the 95th percentile as a cut-off value for aCL and a β_2 GPI in purely obstetric APS, a

small number of false positives are likely to result. However, if the 99th percentile were used as a cut-off in these women, many cases of obstetric APS are likely to be missed. The role of IgM a β_2 GPI testing in the diagnosis of APS remains unclear and it is considered to have little clinical utility for thrombotic APS.³⁸ In our cohort >50% of women with obstetric APS had low-titre IgM aCL and/or a β_2 GPI (alone or in association with IgG antibodies). Given the favourable risk/benefit ratio of heparin/aspirin treatment during pregnancy for obstetric APS it appears reasonable, at present, to offer this treatment during pregnancy to women with low-titre aPL associated with obstetric APS. However, future studies into the clinical significance and management of low-titre aPL in obstetric APS would be useful.

In conclusion, these data suggest that LA, IgG and IgM aCL and a β_2 GPI testing are all required for the accurate diagnosis and assessment of prognosis of APS in routine clinical practice. Furthermore, as laboratory tests for aPL remain poorly standardized internationally, it is prudent to retain testing for LA as well as aCL and a β_2 GPI for APS diagnosis. Our data also suggest that low-titre aCL and a β_2 GPI should be included in the laboratory criteria for diagnosis of purely obstetric APS. This should be validated in a prospective multicentre study.

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Conflict of interest statement

The author has no conflict of interest to declare.

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 Galli M, Reber G, de MP DE, Groot PG. Invitation to a debate on the serological criteria that define the antiphospholipid syndrome. *J Thromb Haemost* 2008; 6: 399–401.
- 3 Galli M, Luciani D, Bertolini G, Barbui T. 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.
- 4 Opatrny L, David M, Kahn SR, Shrier I, Rey E. Association between antiphospholipid antibodies and recurrent fetal loss in women without autoimmune disease: a meta-analysis. *J Rheumatol* 2006; 33: 2214–2221.
- 5 Pengo V, Tripodi A, Reber G, *et al.* Update of the guidelines for lupus anticoagulant detection. Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibody of the Scientific and Standardisation Committee of the International Society on Thrombosis and Haemostasis. *J Thromb Haemost* 2009; 7: 1737–1740.
- 6 Nash MJ, Camilleri RS, Kunka S, Mackie IJ, Machin SJ, Cohen H. The anticardiolipin assay is required for sensitive screening for antiphospholipid antibodies. *J Thromb Haemost* 2004; 2: 1077–1081.
- 7 Boffa MC, Boinot C, De Carolis S, Rovere-Querini P, *et al.* Laboratory criteria of the obstetrical antiphospholipid syndrome. Data from a multicentric prospective European women cohort. *J Thromb Haemost* 2009; 102: 25–28.
- 8 Wahl D, Thiebaugeorges O, Regnault V, Dalloul A, Lecompte T. Pursuing the debate on the serologic criteria that define the antiphospholipid syndrome. *J Thromb Haemost* 2008; 6: 1433–1435.
- 9 Ruffatti A, Olivieri S, Tonello M, Bortolati M, *et al.* Influence of different IgG anticardiolipin antibody cut-off values on antiphospholipid syndrome classification. *J Thromb Haemost* 2008; 6: 1693–1696.
- 10 Cohn DM, Goddijn M, Middeldorp S, Korevaar JC, Dawood F, Farquharson RG. Recurrent miscarriage and antiphospholipid antibodies: prognosis of subsequent pregnancy. *J Thromb Haemost* 2010; 8: 2208–2213.
- 11 Hughes GR, Khamashta MA. Seronegative antiphospholipid syndrome. *Ann Rheum Dis* 2003; 62: 1127.
- 12 Ruiz-Irastorza G, Hunt BJ, Khamashta MA. A systematic review of secondary thromboprophylaxis in patients with antiphospholipid antibodies. *Arthritis Rheum* 2007; 57: 1487–1495.
- 13 Kasthuri RS, Roubey RA. Warfarin and the antiphospholipid syndrome: does one size fit all? *Arthritis Rheum* 2007; 57: 1346–1347.
- 14 McNally T, Purdy G, Mackie IJ, Machin SJ, Isenberg DA. The use of an anti-beta 2-glycoprotein-I assay for discrimination between anticardiolipin antibodies associated with infection and increased risk of thrombosis. *Br J Haematol* 1995; 91: 471–473.
- 15 Loizou S, McCrea JD, Rudge AC, Reynolds R, Boyle CC, Harris EN. Measurement of anti-cardiolipin antibodies by an enzyme-linked immunosorbent assay (ELISA): standardization and quantitation of results. *Clin Exp Immunol* 1985; 62: 738–745.
- 16 Harris EN, Gharavi AE, Patel SP, Hughes GR. Evaluation of the anti-cardiolipin antibody test: report of an international workshop held 4 April 1986. *Clin Exp Immunol* 1987; 68: 215–222.
- 17 Farquharson RG, Quenby S, Greaves M. Antiphospholipid syndrome in pregnancy: a randomized, controlled trial of treatment. *Obstet Gynecol* 2002; 100: 408–413.
- 18 McNally T, Mackie IJ, Machin SJ, Isenberg DA. Increased levels of beta 2 glycoprotein-I antigen and beta 2 glycoprotein-I binding antibodies are associated with a history of thromboembolic complications in patients with SLE and primary antiphospholipid syndrome. *Br J Rheumatol* 1995; 34: 1031–1036.
- 19 Brandt JT, Triplett DA, Alving B, Scharrer I. Criteria for the diagnosis of lupus anticoagulants: an update. On behalf of the Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibody of the Scientific and Standardisation Committee of the ISTH. *Thromb Haemost* 1995; 74: 1185–1190.
- 20 Greaves M, Cohen H, Machin SJ, Mackie I. Guidelines on the investigation and management of the antiphospholipid syndrome. *Br J Haematol* 2000; 109: 704–715.
- 21 Rooney AM, McNally T, Mackie IJ, Machin SJ. The Taipan snake venom time: a new test for lupus anticoagulant. *J Clin Pathol* 1994; 47: 497–501.
- 22 Pengo V, Ruffatti A, Legnani C, *et al.* Clinical course of high-risk patients diagnosed with antiphospholipid syndrome. *J Thromb Haemost* 2010; 8: 237–242.
- 23 Tans G, Rosing J, Thomassen MC, Heeb MJ, Zwaal RF, Griffin JH. Comparison of anticoagulant and procoagulant activities of stimulated platelets and platelet-derived microparticles. *Blood* 1991; 77: 2641–8.
- 24 Ruffatti A, Tonello M, Cavazzana A, Bagatella P, Pengo V. Laboratory classification categories and pregnancy outcome in patients with primary antiphospholipid syndrome prescribed antithrombotic therapy. *Thromb Res* 2009; 123: 482–487.
- 25 Ruffatti A, Tonello M, Del RT, *et al.* Antibody profile and clinical course in primary antiphospholipid syndrome with pregnancy morbidity. *J Thromb Haemost* 2006; 96: 337–341.
- 26 Chaleur C, Galanaud JP, Alonso S, *et al.* Observational study of pregnant women with a previous spontaneous abortion before the 10 gestation week with and without antiphospholipid antibodies. *J Thromb Haemost* 2010; 8: 699–706.
- 27 Greco TP, Conti-Kelly AM, Matsuura E, *et al.* Antiphospholipid antibodies in patients with coronary artery disease: new cardiac risk factors? *Ann N Y Acad Sci* 2007; 1108: 466–474.
- 28 Yamada H, Atsumi T, Kobashi G, *et al.* Antiphospholipid antibodies increase the risk of pregnancy-induced hypertension and adverse pregnancy outcomes. *J Reprod Immunol* 2009; 79: 188–195.
- 29 Rai RS, Clifford K, Cohen H, Regan L. High prospective fetal loss rate in untreated pregnancies of women with recurrent miscarriage and antiphospholipid antibodies. *Hum Reprod* 1995; 10: 3301–3304.
- 30 Rai R, Cohen H, Dave M, Regan L. Randomised controlled trial of aspirin and aspirin plus heparin in pregnant women with recurrent miscarriage associated with phospholipid antibodies (or antiphospholipid antibodies). *BMJ* 1997; 314: 253–257.
- 31 Kutteh WH. Antiphospholipid antibody-associated recurrent pregnancy loss: treatment with heparin and low-dose aspirin is superior to low-dose aspirin alone. *Am J Obstet Gynecol* 1996; 174: 1584–1589.
- 32 Favaloro EJ, Silvestrini R. Assessing the usefulness of anticardiolipin antibody assays: a cautious approach is suggested by high variation and limited consensus in multilaboratory testing. *Am J Clin Pathol* 2002; 118: 548–557.
- 33 Pierangeli SS, Harris EN. A quarter of a century in anticardiolipin antibody testing and attempted standardization has led us to here, which is? *Semin Thromb Hemost* 2008; 34: 313–328.
- 34 Reber G, Schousboe I, Tincani A, *et al.* Inter-laboratory variability of anti-beta2-glycoprotein I measurement. A collaborative study in the frame of the European Forum on Antiphospholipid Antibodies Standardization Group. *J Thromb Haemost* 2002; 88: 66–73.
- 35 Cavazzana A, Pengo V, Tonello M, *et al.* Anti-beta(2)-glycoprotein I ELISA assay: the influence of different antigen preparations. *J Thromb Haemost* 2009; 101: 789–791.
- 36 Brighton TA, Dai YP, Hogg PJ, Chesterman CN. Microheterogeneity of beta-2 glycoprotein I: implications for binding to anionic phospholipids. *Biochem J* 1999; 340: 59–67.
- 37 Bertolaccini ML, Gomez S, Pareja JF, *et al.* Antiphospholipid antibody tests: spreading the net. *Ann Rheum Dis* 2005; 64: 1639–1643.
- 38 Keeling D, Mackie I, Moore GW, Greer IA, Greaves M. Guidelines on the investigation and management of antiphospholipid syndrome. *Br J Haematol* 2012; Feb 8.