

# Diagnosis and management of non-criteria obstetric antiphospholipid syndrome

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## Summary

Accurate diagnosis of obstetric antiphospholipid syndrome (APS) is a prerequisite for optimal clinical management. The international consensus (revised Sapporo) criteria for obstetric APS do not include low positive anticardiolipin (aCL) and anti  $\beta_2$  glycoprotein I (a $\beta_2$ GPI) antibodies (<99<sup>th</sup> centile) and/or certain clinical criteria such as two unexplained miscarriages, three non-consecutive miscarriages, late pre-eclampsia, placental abruption, late premature birth, or two or more unexplained *in vitro* fertilisation failures. In this review we examine the available evidence to address the question of whether patients who exhibit non-criteria clinical and/or laboratory manifestations should be included within the spectrum of obstetric APS. Prospective and retrospective cohort studies of women with pregnancy morbidity, particularly recurrent pregnancy loss, suggest that elimination of aCL and/or IgM a $\beta_2$ GPI, or low positive aCL or a $\beta_2$ GPI from APS laboratory diagnostic criteria may result in missing the diagnosis in a

sizeable number of women who could be regarded to have obstetric APS. Such prospective and retrospective studies also suggest that women with non-criteria obstetric APS may benefit from standard treatment for obstetric APS with low-molecular-weight heparin plus low-dose aspirin, with good pregnancy outcomes. Thus, non-criteria manifestations of obstetric APS may be clinically relevant, and merit investigation of therapeutic approaches. Women with obstetric APS appear to be at a higher risk than other women of pre-eclampsia, placenta-mediated complications and neonatal mortality, and also at increased long-term risk of thrombotic events. The applicability of these observations to outcomes in women with non-criteria obstetric APS remains to be determined.

## Keywords

Antiphospholipid syndrome, international consensus criteria, obstetric APS, low titre aPL, recurrent miscarriages, heparin, aspirin

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## Introduction

The antiphospholipid syndrome (APS) is characterised by vascular thrombosis and/or pregnancy complications, in association with persistently positive antiphospholipid antibodies (aPL). The international consensus (revised Sapporo) criteria for APS-related pregnancy morbidity, which may occur alone (obstetric APS) or in combination with thrombotic manifestations, summarised in ► Table 1A, are as follows: a) one or more unexplained deaths of a morphologically normal fetus at or beyond the 10th week of gestation, with normal fetal morphology documented by ultrasound or by direct examination of the fetus; or b) one or more premature births of a morphologically normal neonate before the 34th week of gestation (because of: i) eclampsia or severe pre-eclampsia defined according to standard definitions; ii) recognised features of placental insufficiency); or c) three or more unexplained consecutive spontaneous miscarriages before the 10th week of gestation (i.e. recurrent miscarriage), with maternal anatomic or hormonal abnormalities and paternal and maternal chromosomal causes excluded) (1, 2). Recurrent miscarriage affects 1% of all women (3). Known risk factors include chromosomal abnormalities, which is probably the common-

est cause, with other causes including anatomical or hormonal disorders, and APS (4, 5), with the cause unexplained in over 50% of cases (6, 7). Major determinants of the prognosis include whether or not an underlying cause is found as well as maternal age and the number of preceding miscarriages (8). The international consensus laboratory criteria for APS are the presence of: aPL, i.e. lupus anticoagulant (LA); and/or moderate or high positive IgG or IgM anticardiolipin (aCL) (i.e. >40GPL or MPL or >99<sup>th</sup> centile); and/or anti- $\beta_2$ glycoprotein-1 (a $\beta_2$ GPI) (IgG and/or IgM) antibodies >99<sup>th</sup> centile. Persistently positive aPL is defined as detection on two or more consecutive occasions at least 12 weeks apart (2).

Accurate diagnosis of obstetric APS is a prerequisite for optimal clinical management, and thereby, the potential prevention of long-term disability of the offspring as a result of placenta-mediated obstetric morbidity such as intrauterine growth restriction (IUGR), early onset pre-eclampsia or placental insufficiency (9, 10). In addition, recurrent miscarriages are associated with significant psychological sequelae for both women and their partners, with anxiety, depression, denial, anger, marital disruption, and a sense of loss and inadequacy being common (11). The original purpose of the international consensus (revised Sapporo) criteria

Clinical criteria	Laboratory criteria
<ol style="list-style-type: none"> <li>One or more unexplained deaths of a morphologically normal fetus at or beyond the 10th week of gestation</li> <li>One or more pre-term births of a morphologically normal neonate before the 34th week of gestation because of: <ul style="list-style-type: none"> <li>(i) eclampsia or severe pre-eclampsia or</li> <li>(ii) recognised features of placental insufficiency</li> </ul> </li> <li>Three or more unexplained consecutive spontaneous miscarriages before the 10th week of gestation, with maternal anatomic or hormonal abnormalities and paternal and maternal chromosomal causes excluded</li> </ol>	<ol style="list-style-type: none"> <li>LA present in plasma, on two or more occasions at least 12 weeks apart</li> <li>aCL of immunoglobulin (Ig)G and/or IgM isotype in serum or plasma, present in medium or high titre (i.e. &gt;40GPL units or MPL units, or &gt; the 99th centile), on two or more occasions, at least 12 weeks apart</li> <li>a<math>\beta</math>2GPI of IgG and/or IgM isotype in serum or plasma (in titre &gt;the 99th centile), present on two or more occasions at least 12 weeks apart</li> </ol>
OAPS is diagnosed if at least one of the clinical criteria and one of the laboratory criteria are met	
OAPS: Obstetric antiphospholipid syndrome; LA: lupus anticoagulants; aCL: anticardiolipin antibodies; a $\beta$ 2GPI: anti $\beta$ 2glycoprotein-I antibodies.	

**Table 1A: The international consensus (revised Sapporo) criteria for diagnosis of obstetric antiphospholipid syndrome.**

Clinical criteria	Laboratory criteria
<ol style="list-style-type: none"> <li>Two unexplained miscarriages</li> <li>Three non-consecutive miscarriages</li> <li>Late pre-eclampsia</li> <li>Placental abruption, late premature birth,</li> <li>Two or more unexplained <i>in vitro</i> fertilisation failures</li> </ol>	<ol style="list-style-type: none"> <li>Low positive aCL or a<math>\beta</math>2GPI present between the 95th and 99th centiles</li> <li>Presence of intermittent aPL in women with classical clinical manifestations of obstetric APS.</li> </ol>
A diagnosis of non-criteria obstetric APS is considered to be present if the patient has: a) a combination of non-criteria clinical manifestations with international consensus laboratory criteria; or b) international consensus clinical criteria with a non-criteria laboratory manifestation.	
aCL: anticardiolipin antibodies; a $\beta$ 2GPI: anti $\beta$ 2glycoprotein-I antibodies; aPL: antiphospholipid antibodies; OAPS: obstetric antiphospholipid syndrome.	

**Table 1B: Non-criteria clinical and laboratory manifestations of obstetric antiphospholipid syndrome.**

was to standardise multi-centre studies and clinical trials in APS, and they were not intended to be used for diagnostic purposes in routine clinical practice. However, it is useful to have firm, evidence based, diagnostic criteria for routine clinical use, which may differ from those defined for clinical studies.

The British Committee for Standards in Haematology (BCSH) guidelines (12), based on the international consensus criteria, adopt similar criteria for the diagnosis of both thrombotic and obstetric APS. The international consensus criteria for obstetric APS do not include low positive anticardiolipin (aCL) and anti  $\beta$ 2 glycoprotein I (a $\beta$ 2GPI) antibodies (<99th centile) and/or certain clinical criteria such as two unexplained miscarriages, three non-consecutive miscarriages, late pre-eclampsia, placental abruption, late premature birth, or two or more unexplained *in vitro* fertilisation failures. Evidence is accumulating on the potential clinical significance of these non-criteria clinical and laboratory manifestations of obstetric APS (8, 10, 13, 14) (► Table 1B). In this review we examine the available evidence to address the question of whether patients who exhibit non-criteria clinical and/or laboratory manifestations should be included within the spectrum of obstetric APS.

## Search criteria

A literature search was carried out on PubMed for publications in the last 30 years using the following phrases: antiphospholipid syn-

drome, international consensus criteria, obstetric APS, low titre antiphospholipid antibodies, recurrent miscarriages, pregnancy morbidity, non-criteria aPL, non-criteria obstetric morbidity, guidelines on management of obstetric APS, heparin, low-molecular-weight heparin (LMWH) and aspirin.

## Thrombotic and obstetric APS

APS is generally considered to be a thromboembolic disease, with long-term anticoagulation with vitamin K antagonists such as warfarin the current mainstay of treatment. APS patients with systemic thromboembolism may also develop thrombosis in the placental vasculature leading to obstetric morbidity such as IUGR (15–17). A small percentage of women with pure obstetric APS subsequently develop thrombosis. In a multicentre prospective study of 1,000 patients, Cervera et al. studied the morbidity and mortality in APS during a five-year period. They found that most women with APS-related pregnancy complications do not have thrombosis or other manifestations of the syndrome at presentation, and only infrequently (i.e. in less than 5% of patients) progress to develop proven thrombosis or systemic lupus erythematosus (SLE) (18). However, the Nimes Obstetricians and Haematologists Antiphospholipid Syndrome (NOH-APS) observational study reported that the annual rates of deep-vein thrombosis (1.46%; range 1.15%–1.82%), pulmonary embolism (0.43%; range 0.26%–0.66%), superficial

vein thrombosis (0.44%; range 0.28%–0.68%), and cerebrovascular events (0.32%; range 0.18%–0.53%) were significantly higher in women with pure obstetric APS compared with aPL-negative women with obstetric morbidity, despite low-dose aspirin (LDA) primary prophylaxis (19). The applicability of these observations to long-term outcomes in women with non-criteria obstetric APS remains to be determined.

aPL are associated with an increased risk of recurrent and late pregnancy loss, with approximately 15% of women with recurrent miscarriage reported to have aPL (20, 21). An analysis by the Anti-Phospholipid Syndrome Alliance For Clinical Trials and International Networking (APS ACTION), based on 120 full-text papers and calculation of the median frequency for positive aPL tests for clinical outcome, estimated the overall frequency of aPL in pregnancy morbidity to be 6% (interquartile range 2–13%) (22). A meta-analysis of the association between aPL and recurrent fetal loss in women without autoimmune disease, reported that LA, which appears to have the strongest association with thrombosis (23), was associated with late recurrent pregnancy loss (odds ratio [OR] 7.79, 95% confidence interval [CI] 2.30–26.45), by which time the placental circulation is well formed. The data were insufficient to enable analysis of the association of LA with early miscarriages (<13 weeks). IgG aCL, both low and moderate to high antibody levels, were associated with both early (OR 3.56, 95% CI 1.48–8.59) and late recurrent pregnancy loss (OR 3.57, 95% CI 2.26–5.65). Restriction of the analysis to include only women with moderate to high aPL levels (>99th centile) increased the strength of the association (OR 4.68, 95% CI 2.96–7.40). IgM aCL were also associated with late recurrent fetal loss (OR 5.61, 95% CI 1.26–25.03). No association was found between early recurrent pregnancy loss and a $\beta$ 2GPI (OR 2.12, 95% CI 0.69–6.53) (24).

## Pathogenesis of pregnancy complications in APS

The laboratory findings and the immunopathology of obstetric APS differs from that in thrombotic APS, especially in the scenario of recurrent early miscarriages, where thrombosis is neither a universal nor a specific feature (25).

LA may be associated with extensive placental necrosis, infarction and thrombosis in women with recurrent pregnancy loss. These abnormalities may result from thrombosis during the development of the normal materno-placental circulation, perhaps via interference with trophoblastic annexin V (26). During differentiation to syncytium, trophoblasts express cell membrane anionic phospholipids that can bind  $\beta$ 2GPI. aPL which are  $\beta$ 2GPI-dependent may recognise their own antigen on trophoblast and decidual cells as a 'planted antigen' and it has been suggested that the binding to this antigen affects several trophoblast cell functions, leading to defective placentation (27, 28). In addition, the  $\beta$ 2GPI/anti- $\beta$ 2GPI complex formation may activate complement and thereby induce local inflammatory damage (29).

Complement activation by aPL appears to play a major role in the pathogenesis of recurrent pregnancy loss, and there is some

evidence that complement activation may also have a role in the pathogenesis of thrombosis in APS (30). Appropriate complement inhibition is an essential requirement for normal pregnancy; this is supported by the finding that deficiency of Crry (a membrane-bound complement regulatory protein, like DAF and MCP that block C3 and C4 activation), leads to progressive embryonic loss in mice (31). It has been hypothesised that aPL bound to trophoblasts activate complement via the classical pathway, generating split products that mediate placental injury and play a causative role in fetal loss and growth restriction. Support for this hypothesis comes from studies in a murine model of APS. These demonstrate that in pregnant mice injected with human IgG containing aPL antibodies, inhibition of the complement cascade *in vivo*, using the C3 convertase inhibitor to complement receptor 1-related gene/protein (Crry)-Ig, blocks fetal loss and growth restriction. It has also been demonstrated that antibodies or peptides that block C5a-C5a receptor interactions prevent pregnancy complications. Studies in factor B-deficient mice, however, indicate that alternative pathway activation is required and amplifies complement activation. Furthermore, mice deficient in complement C3 are resistant to fetal injury induced by aPL (32, 33). Passive transfer of IgG from women with recurrent miscarriage and aPL results in a significant increase in the frequency of fetal resorption and reduced average weight of the surviving fetuses, compared to mice treated with IgG from healthy individuals (33). It has been suggested that heparin prevents obstetric complications caused by aPL, because it blocks complement activation rather than through its antithrombotic properties (34). These findings further support the view that at least some of the pathogenic mechanisms for obstetric manifestations of APS may differ from thrombotic APS. It follows therefore that it is plausible that there may be differences in aPL phenotypes in these conditions.

## Non-criteria obstetric APS: clinical manifestations

Several obstetric manifestations additional to those in the international consensus criteria have been proposed as 'obstetric morbidity associated with APS (OMAPS)' (14). These include two unexplained miscarriages, three non-consecutive miscarriages, late pre-eclampsia, placental abruption, late premature birth, or two or more unexplained *in vitro* fertilisation failures. Late pregnancy complications such as severe pre-eclampsia and placental insufficiency at less than 34 weeks gestation are relatively common, probably complicating about 0.5% to 1% of pregnancies and thought to be multifactorial. The preliminary first year report from the European Registry on Obstetric Antiphospholipid Syndrome (EU-ROAPS) suggested that there were no statistically significant differences in pregnancy outcome between women with obstetric APS, as defined by the International consensus criteria, or OMAPS. Overall, live births were obtained in 79.4% (154/194) patients with obstetric APS and 93.7% (30/32) patients with OMAPS (14). Such studies also suggest that women with non-criteria obstetric APS may benefit from standard treatment for obstetric APS with LMWH plus LDA, with good pregnancy outcomes. Thus, non-criteria manifestations of obstetric APS may be clinically rel-

evant, and merits investigation of therapeutic approaches. Robertson et al. (21) noted the paucity of prospective studies that have addressed the possible association of APS with severe pre-eclampsia or placental insufficiency requiring delivery prior to 34 weeks gestation. A study by Branch et al. (35) concluded that testing for aPL during pregnancy is of little prognostic value in the assessment of the risk for recurrent preeclampsia among women with a history of preeclampsia. The Obstetric Task Force at of the 13<sup>th</sup> International Congress on Antiphospholipid Antibodies noted that despite inclusion of fetal death, placental insufficiency and severe early pre-eclampsia in the international consensus criteria for diagnosis of obstetric APS, the data supporting the associations have been conflicting to date and there is a lack of robust evidence to guide treatment (36). More recently, the Nimes Obstetricians and Haematologists Antiphospholipid Syndrome the (NOH-APS) observational study (37) found that among women with a history of recurrent miscarriages, those with APS were at a higher risk of pre-eclampsia, placenta-mediated complications, and neonatal mortality. Among women with prior fetal loss, LMWH and LDA-treated women had lower pregnancy loss rates but higher pre-eclampsia rates than aPL-negative women. The applicability of these observations to pregnancy outcomes in women with non-criteria obstetric APS remains to be determined.

### Non-criteria obstetric APS: laboratory manifestations

The majority of the published studies do not document the prevalence of a $\beta$ 2GPI positivity during the normal child bearing years as

these studies (38–40) were generally undertaken prior to the inclusion of a $\beta$ 2GPI in the international consensus laboratory criteria (1, 2). This makes it difficult to distinguish whether the presence of low positive a $\beta$ 2GPI in women with obstetric morbidity is causal or incidental. The specificity of recurrent early miscarriages is probably low due to difficulty in fully excluding other potential causes (2), the commonest of these being a chromosomal abnormality (7, 41).

Galli et al. have argued that both IgG and IgM aCL and IgM a $\beta$ 2GPI should be dropped from the laboratory criteria for APS and only IgG a $\beta$ 2GPI and LA should be considered (42). Conversely, several experts propose that omission of all aCL testing from the clinical investigation of APS would lead to a failure to diagnose the syndrome in a significant proportion of women with obstetric APS (43–45). Although triple positivity (the co-existence of LA, aCL and a $\beta$ 2GPI) is associated with a significantly higher rate of pregnancy loss in obstetric APS (46), a single positive aPL test, more often aCL or a $\beta$ 2GPI alone rather than LA, has been reported to be more common in purely obstetric APS (10, 19). This was the case in a multicentre, prospective European cohort in which out of 109 pregnant women with APS (73 women had purely obstetric APS, unassociated with other autoimmune diseases or thrombosis), 46% (50/109) exhibited a single positive antibody (either aCL or a $\beta$ 2GPI) and 31% (34/109) aCL alone (45). In the NOH-APS observational study, positivity for IgM aCL was an independent risk factor for placental-mediated complications. Positive results for LA, IgG a $\beta$ 2GPI or aCL in treated APS women did not indicate significant risks and a triple positivity pattern had no predictive value (37).

**Table 2: Studies demonstrating the significance of including low positive aPL (aCL and a $\beta$ 2GPI) in diagnosing obstetric APS.**

Reference and year	Number of patients with obstetric APS	Laboratory criteria used to diagnose obstetric APS	Repeat testing
Retrospective observational cohort study (8) 2010	176/693 (25%)	aCL levels > 95th centile healthy adults a $\beta$ 2GPI were not tested	Yes (100%) 6 or more weeks apart
Retrospective Cohort study (13) 2012	As per ICS criteria 23/25 (92%) Low aPL 27/ 32 (84%) had only APS-like obstetrical events	1. ICS laboratory criteria for aCL and a $\beta$ 2GPI 2. Low titre IgG/IgM a $\beta$ 2GPI and/or IgG/IgM aCL (> 90th<99th percentile)	Yes (100%) 12 weeks apart
Prospective registry (14) 2012	194/226 (85.8%) OAPS 32/226 (14.6%) OMAPS.	1. ICS laboratory criteria 2. Non-standard aPL: anti-annexin A5, aPS, aPS/PT or other aPL. 3. ICS or non-standard aPL, detected less than 12 weeks apart, on two occasions. 4. ICS or non-standard antibodies just described above, detected only on one occasion, especially in gestational time. 5. Low levels of aCL (IgG/IgM) and/or a $\beta$ 2GPI (levels<99 centile)	Yes (89%) 12 weeks apart or detected once
Retrospective audit (10) 2013	40/145 (28%)	1. ICS laboratory criteria for aCL and a $\beta$ 2GPI 2. Low titre IgG/IgM aCL I and/or IgG/IgM a $\beta$ 2GPI (> 95th<99th percentile) Reference ranges for aCL and a $\beta$ 2GPI were obtained from 240 normal healthy volunteers	Yes (100%) 12 weeks apart

APS: Antiphospholipid syndrome; aCL: anticardiolipin antibodies; a $\beta$ 2GPI: anti- $\beta$ 2glycoprotein-1 antibodies; ICS: international consensus; OAPS: Obstetric antiphospholipid syndrome; OMAPS: obstetric morbidity associated to antiphospholipid antibody syndrome; aPS: antiphosphatidylserine; aPS/PT: anti-PS/prothrombin, LMWH: low molecular weight heparin; LDA : low dose aspirin.

Both retrospective and prospective studies of women with pregnancy morbidity, particularly recurrent pregnancy loss, suggest that elimination of aCL and IgM a $\beta$ 2GPI from APS laboratory diagnostic criteria may result in missing the diagnosis in a sizeable number of women who could be regarded to have obstetric APS (► Table 2). The cut-off value for positivity in aCL and a $\beta$ 2GPI assays is also under debate. Several studies suggest that women with isolated obstetric APS may have lower aCL levels than patients with a thrombotic history. Ruffatti et al. (47) reported that the rate of aCL values between the 99th centile (17.4 for IgG and 26.8 for IgM aCL) 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%). Gardiner et al., (10) demonstrated that over 50% of women with clinical features of obstetric APS, but no thrombosis, had low positive aCL and/or a $\beta$ 2GPI in the absence of LA. Detection of positive or negative LA might depend on how LA testing is performed (i.e. which combination of tests is employed), for example the activated partial thromboplastin time and kaolin clotting time or kaolin cephalin clotting time would detect anti-FXII, which appears to be more frequent in obstetric APS (48), whereas the dilute prothrombin time and dilute Russell's viper venom time test might be more sensitive to prothrombin antibodies (49). In the study by Gardiner et al., (10) IgG/IgM aCL levels and IgM a $\beta$ 2GPI levels were significantly higher in patients with a history of thrombosis than in women with a history of purely obstetric APS ( $p < 0.05$ ), while the rate of LA positivity was significantly higher in patients with a history of thrombosis compared with those with obstetric APS alone (50.5% vs 15%;  $p < 0.0002$ ). This study suggested that the omission of aCL or a $\beta$ 2GPI testing from the investigation of APS would have led to a failure to diagnose APS in 9.5% and 29.4% of patients, respectively (10). Studies of aPL phenotype in obstetric APS have several limitations. The majority of the studies include retrospective cohort studies with a small number (25–176) of selected patients (10; 13) and prospective registry with 226 patients (14). Different methods have been used to detect aCL and a $\beta$ 2GPI antibodies, which makes it challenging to compare results from different studies. There are now various guidelines that recommend which assays to use and guide standardisation of the methodology (50–53). A wide variation exists in the determination of reference ranges of aCL and a $\beta$ 2GPI in normal populations. Reference ranges for aCL and a $\beta$ 2GPI antibodies should be established by the non-parametric centile method, as autoantibody values are usually not normally distributed (51–54). International consensus guidelines state that at least 120 normal subjects are required to establish an accurate reference range (taking into consideration the age and the type of population most representative for each laboratory) (50). The number of healthy volunteers studied to obtain reference ranges for aCL and a $\beta$ 2GPI varied in the studies cited above from 50 to 240, which would affect the cut-off values and hence the number/percentage of women diagnosed as obstetric APS. The study by Mekinian et al. to investigate the outcomes and treatment of obstetric APS in women with low aPL levels included those who had aCL and a $\beta$ 2GPI levels between the 90th and 99th centile (13). This group was compared with those

women who had aCL and a $\beta$ 2GPI levels above 99th centile. The total number of obstetric events per patient was similar before treatment with LMWH plus LDA and decreased significantly after treatment with LMWH plus LDA in the two groups. Overall, there is no concordance as to the level of aCL or a $\beta$ 2GPI used to define low aPL levels. Data from a retrospective cohort study (45), and also in the prospective European cohort (14) suggest that low positive aCL should be defined as those between the 95th and 99th centiles and these levels should be used as laboratory criteria to diagnose pure obstetric APS rather than the 99th centile as suggested by the International consensus criteria. The interval of 12 weeks between aPL tests, which appears to be an arbitrary interval, to establish persistence of aPL, may be challenging to achieve in the non-pregnant state in women with obstetric morbidity. This may be compounded by findings suggesting that aPL testing may not be representative during pregnancy (55, 56).

In addition, there are women who are strongly suspected of having obstetric APS, showing the classical clinical features but persistently negative for currently recommended laboratory tests for aPL. It has been proposed that autoantibodies directed against negatively charged phospholipids other than cardiolipin; other proteins of coagulation cascade; specific domains of  $\beta$ 2GPI, or those interfere with the anticoagulant activity of annexin A5; may be to relevant to APS and defined as non-criteria aPL (57, 58). Some authors have described this phenomenon as 'seronegative APS' (59). These patients were often positive for antibodies to zwitterionic phospholipid (e.g. phosphatidylethanolamine); various phospholipid-binding plasma proteins / phospholipid-protein complexes; and anionic phospholipids other than cardiolipin. However, clinical significance of these antibodies is unclear and the assays need further standardisation before their presence and prevalence can be confirmed and their clinical relevance investigated in large multicentre, prospective studies.

## Treatment of obstetric APS

There are few prospective randomised controlled trials (RCT) in women with aPL and a history of recurrent miscarriages. Rai et al. showed a significant increase in live births following treatment with LDA plus unfractionated heparin (UFH) (71%) vs LDA alone (40%) (60). The majority of the women in this study had only LA as the laboratory criteria for APS (34/45 women in the LDA arm and 40/45 women in the UFH plus LDA arm had only LA) (60). Concordant findings were observed in a RCT by Goel et al. (61) involving 72 patients with two or more spontaneous miscarriages and IgG aCL, when treated with a combination of UFH and LDA compared with LDA alone. Conversely, Farquharson et al. (62) reported that the addition of LMWH to LDA did not significantly improve pregnancy outcome compared to LDA alone. Limited data suggest that persistent weak aCL (<99th centile) in untreated pregnancies of women with recurrent miscarriage is associated with a fetal loss rate of over 90% (63).

In a meta-analysis of data from five trials involving 334 patients with recurrent miscarriage (64), the overall live birth rates were

74.3 and 55.9% in women who received a combination of UFH/LMWH plus LDA vs that in those treated with LDA alone. Patients who received combination treatment had significantly higher live birth rates (relative risk [RR] 1.301; 95% CI 1.040, 1.629) than with aspirin alone. No significant differences in pre-eclampsia, preterm labour and birth weight were found between two groups. In accordance with these observations, both the BCSH (12) and American College of Chest Physicians (ACCP) (65) guidelines provide recommendations on the management of women who fulfil the clinical and laboratory international consensus criteria for obstetric APS, based on a history of recurrent miscarriages. The BCSH guidance is that such women should be screened for aPL, and in women with APS, antenatal administration of heparin combined with LDA is recommended throughout pregnancy, in general starting as soon as pregnancy is confirmed and continuing until six weeks post-partum. The ACCP guidelines also recommend that these women with obstetric APS should be treated with prophylactic or intermediate dose unfractionated heparin (UFH) or prophylactic dose LMWH combined with LDA (75 to 100 mg/daily), in the antepartum period as soon as pregnancy is confirmed. In practice, most clinicians favour LMWH. The ACCP guidelines recommend LDA alone throughout pregnancy, starting from the second trimester for women considered at risk for preeclampsia. The BCSH guidelines also recommend LDA alone for women with APS and a history of pre-eclampsia.

Prospective (14) and retrospective (8; 10; 13) cohort studies in patients with non-criteria laboratory manifestations of obstetric APS suggest that they may have similar pregnancy outcomes with standard treatment for obstetric APS as women who fulfil international consensus criteria for obstetric APS. In a retrospective observational cohort study by Farquharson's group (8), 79% of 176 women with obstetric APS (diagnosed using aCL cut-off values equal to or higher than log-transformed mean plus two SDs of results in 50 healthy adults), who received LDA and LMWH during their pregnancy had a live birth, compared to 62% of women with aPL who received LDA only (adjusted OR 2.7, 95% CI 1.3–5.8). Although direct comparison studies are lacking, LMWH has superseded the use of UFH in pregnancy (ACCP 2012) because of safety and convenience (65).

## Conclusions

APS probably constitutes the single most recognisable risk factor in the majority of cases of recurrent pregnancy loss and late placenta-mediated obstetric morbidity. The pathogenic mechanisms underlying pregnancy complications in women with APS may differ from those in thrombotic APS. Based on several randomised controlled trials in women with obstetric APS, the BCSH and ACCP (12, 65) recommendations support that women with obstetric APS manifested as recurrent miscarriage, who meet International consensus criteria, should be treated with prophylactic or intermediate dose UFH or prophylactic LMWH combined with LDA, in the antepartum period as soon as pregnancy is confirmed.

Prospective (14) and retrospective (8, 10, 13) cohort studies of women with pregnancy morbidity, particularly recurrent pregnan-

cy loss, suggest that elimination of aCL and/or IgM a $\beta$ 2GPI, or low positive aCL or a $\beta$ 2GPI from APS laboratory diagnostic criteria may result in missing the diagnosis in a sizeable number of women who could be regarded to have obstetric APS. Such studies also suggest that women with non-criteria, clinical and/or laboratory, obstetric APS ('obstetric morbidity associated with APS (OMAPS)') may benefit from standard treatment for obstetric APS with LMWH plus LDA, with good pregnancy outcomes. Women with obstetric APS appear to be at higher risk than other women of pre-eclampsia, placenta-mediated complications and neonatal mortality. Accurate diagnosis of obstetric APS is a prerequisite for optimal clinical management, and thereby, the potential prevention of long-term disability as a result of placenta-mediated obstetric complications. Women with obstetric APS also appear to be at increased long-term risk of thrombotic events. The applicability of these observations to outcomes in women with non-criteria obstetric APS remains to be determined.

Prospective multicentre studies, appropriately designed and adequately powered, are required to investigate the diagnostic validity, management implications and long-term outcomes of non-criteria clinical and/or laboratory manifestations of obstetric APS. These studies are difficult to undertake but ultimately the optimal way forward. In the meantime, decisions about the use of anti-thrombotic therapy during pregnancy in women with non-criteria clinical and/or laboratory manifestations of obstetric APS, should be based on an individual risk/benefit assessment. Management should ideally be within a high risk antenatal clinic setting and treatment decisions discussed with the patient and documented.

## Conflicts of interest

None declared.

## References

1. Wilson WA, et al. International consensus statement on preliminary classification criteria for definite antiphospholipid syndrome: report of an international workshop. *Arthritis Rheum* 1999; 42: 1309–1311.
2. Miyakis S, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J Thromb Haemost* 2006; 4: 295–306.
3. Stirrat GM. Recurrent miscarriage. *Lancet* 1990; 336: 673–675.
4. Horne AW, Alexander CI. Recurrent miscarriage. *J Fam Plann Reprod Health Care* 2005; 31: 103–107.
5. Rai R, Regan L. Recurrent miscarriage. *Lancet* 2006; 368: 601–611.
6. Porter TF, Scott JR. Evidence-based care of recurrent miscarriage. *Best Pract Res Clin Obstet Gynaecol* 2005; 19: 85–101.
7. Stephenson MD. Frequency of factors associated with habitual abortion in 197 couples. *Fertil Steril* 1996; 66: 24–29.
8. Cohn DM, et al. Recurrent miscarriage and antiphospholipid antibodies: prognosis of subsequent pregnancy. *J Thromb Haemost* 2010; 8: 2208–2213.
9. Boffa MC, et al. European registry of babies born to mothers with antiphospholipid syndrome: a result update. *Lupus* 2009; 18: 900–904.
10. Gardiner C, et al. Diagnosis of antiphospholipid syndrome in routine clinical practice. *Lupus* 2013; 22: 18–25.
11. Craig M, et al. Psychiatric morbidity among patients with recurrent miscarriage. *J Psychosom Obstet Gynaecol* 2002; 23: 157–164.
12. Keeling D, et al. Guidelines on the investigation and management of antiphospholipid syndrome. *Br J Haematol* 2012; 157: 47–58.
13. Mekinian A, et al. Outcomes and treatment of obstetrical antiphospholipid syndrome in women with low antiphospholipid antibody levels. *J Reprod Immunol* 2012; 94: 222–226.

14. Alijotas-Reig J, Ferrer-Oliveras R. The European Registry on Obstetric Antiphospholipid Syndrome (EUROAPS): a preliminary first year report. *Lupus* 2012; 21: 766–768.
15. Rand JH, et al. Pregnancy loss in the antiphospholipid-antibody syndrome—a possible thrombogenic mechanism. *N Engl J Med* 1997; 337: 154–160.
16. Sammaritano LR, et al. Phospholipid binding of antiphospholipid antibodies and placental anticoagulant protein. *J Clin Immunol* 1992; 12: 27–35.
17. Magid MS, et al. Placental pathology in systemic lupus erythematosus: a prospective study. *Am J Obstet Gynecol* 1998; 179: 226–234.
18. Cervera R, et al. Morbidity and mortality in the antiphospholipid syndrome during a 5-year period: a multicentre prospective study of 1000 patients. *Ann Rheum Dis* 2009; 68: 1428–1432.
19. Gris JC, et al. Comparative incidence of a first thrombotic event in purely obstetric antiphospholipid syndrome with pregnancy loss: the NOH-APS observational study. *Blood* 2012; 119: 2624–2632.
20. Rai RS, et al. Antiphospholipid antibodies and beta 2-glycoprotein-I in 500 women with recurrent miscarriage: results of a comprehensive screening approach. *Hum Reprod* 1995; 10: 2001–2005.
21. Robertson L, et al. Thrombophilia in pregnancy: a systematic review. *Br J Haematol* 2006; 132: 171–196.
22. Andreoli L, et al. Estimated frequency of antiphospholipid antibodies in patients with pregnancy morbidity, stroke, myocardial infarction, and deep vein thrombosis: a critical review of the literature. *Arthritis Care Res (Hoboken)* 2013; 65: 1869–1873.
23. Galli M, 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.
24. Opatrny L, et al. Association between antiphospholipid antibodies and recurrent fetal loss in women without autoimmune disease: a metaanalysis. *J Rheumatol* 2006; 33: 2214–2221.
25. Out HJ, et al. Histopathological findings in placentae from patients with intrauterine fetal death and anti-phospholipid antibodies. *Eur J Obstet Gynecol Reprod Biol* 1991; 41: 179–186.
26. Rand JH, et al. Pregnancy loss in the antiphospholipid-antibody syndrome—a possible thrombogenic mechanism. *N Engl J Med* 1997; 337: 154–160.
27. Agostinis C, et al. In vivo distribution of beta2 glycoprotein I under various pathophysiological conditions. *Blood* 2011; 118: 4231–4238.
28. La RL, et al. Beta 2 glycoprotein I and placental anticoagulant protein I in placentae from patients with antiphospholipid syndrome. *J Rheumatol* 1994; 21: 1684–1693.
29. Girardi G. Role of tissue factor in the maternal immunological attack of the embryo in the antiphospholipid syndrome. *Clin Rev Allergy Immunol* 2010; 39: 160–165.
30. Breen KA, et al. Complement activation in patients with isolated antiphospholipid antibodies or primary antiphospholipid syndrome. *Thromb Haemost* 2012; 107: 423–429.
31. Xu C, et al. A critical role for murine complement regulator crry in fetomaternal tolerance. *Science* 2000; 287: 498–501.
32. Girardi G, et al. Complement C5a receptors and neutrophils mediate fetal injury in the antiphospholipid syndrome. *J Clin Invest* 2003; 112: 1644–1654.
33. Holers VM, et al. Complement C3 activation is required for antiphospholipid antibody-induced fetal loss. *J Exp Med* 2002; 195: 211–220.
34. Girardi G, et al. Heparin prevents antiphospholipid antibody-induced fetal loss by inhibiting complement activation. *Nat Med* 2004; 10: 1222–1226.
35. Branch DW, et al. Antiphospholipid antibodies in women at risk for preeclampsia. *Am J Obstet Gynecol* 2001; 184: 825–832.
36. Branch W. Report of the Obstetric APS Task Force: 13th International Congress on Antiphospholipid Antibodies, 13th April 2010. *Lupus* 2011; 20: 158–164.
37. Bouvier S, et al. Comparative incidence of pregnancy outcomes in treated obstetric antiphospholipid syndrome: the NOH-APS observational study. *Blood* 2014; 123: 404–413.
38. Laskin CA, et al. Low molecular weight heparin and aspirin for recurrent pregnancy loss: results from the randomized, controlled HepASA Trial. *J Rheumatol* 2009; 36: 279–287.
39. van den Boogaard E, et al. Number and sequence of preceding miscarriages and maternal age for the prediction of antiphospholipid syndrome in women with recurrent miscarriage. *Fertil Steril* 2013; 99: 188–192.
40. 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.
41. Porter TF, Scott JR. Evidence-based care of recurrent miscarriage. *Best Pract Res Clin Obstet Gynaecol* 2005; 19: 85–101.
42. Galli M, et al. Invitation to a debate on the serological criteria that define the antiphospholipid syndrome. *J Thromb Haemost* 2008; 6: 399–401.
43. Nash MJ, et al. The anticardiolipin assay is required for sensitive screening for antiphospholipid antibodies. *J Thromb Haemost* 2004; 2: 1077–81.
44. Wahl D, et al. Pursuing the debate on the serologic criteria that define the antiphospholipid syndrome. *J Thromb Haemost* 2008; 6: 1433–1435.
45. Boffa MC, et al. Laboratory criteria of the obstetrical antiphospholipid syndrome. Data from a multicentric prospective European women cohort. *Thromb Haemost* 2009; 102: 25–28.
46. Ruffatti A, et al. Antibody profile and clinical course in primary antiphospholipid syndrome with pregnancy morbidity. *Thromb Haemost* 2006; 96: 337–341.
47. Ruffatti A, et al. Influence of different IgG anticardiolipin antibody cut-off values on antiphospholipid syndrome classification. *J Thromb Haemost* 2008; 6: 1693–1696.
48. Jones DW, et al. Reduced factor XII levels in patients with the antiphospholipid syndrome are associated with antibodies to factor XII. *Br J Haematol* 2000; 110: 721–726.
49. Fleck RA, et al. Anti-prothrombin antibodies and the lupus anticoagulant. *Blood* 1988; 72: 512–519.
50. Lakos G, et al. International consensus guidelines on anticardiolipin and anti-beta2-glycoprotein I testing: report from the 13th International Congress on Antiphospholipid Antibodies. *Arthritis Rheum* 2012; 64: 1–10.
51. Pierangeli SS, Harris EN. A protocol for determination of anticardiolipin antibodies by ELISA. *Nat Protoc* 2008; 3: 840–848.
52. Wong RC, et al. Consensus guidelines on anti-cardiolipin antibody testing and reporting. *Pathology* 2004; 36: 63–68.
53. Wong RC, et al. Consensus guidelines on anti-beta 2 glycoprotein I testing and reporting. *Pathology* 2008; 40: 58–63.
54. Tincani A, et al. Minimal requirements for antiphospholipid antibodies ELISAs proposed by the European Forum on antiphospholipid antibodies. *Thromb Res* 2004; 114: 553–558.
55. Topping J, et al. Marked variation in antiphospholipid antibodies during pregnancy: relationships to pregnancy outcome. *Hum Reprod* 1999; 14: 224–228.
56. Kwak JY, et al. Biological basis of fetoplacental antigenic determinants in the induction of the antiphospholipid antibody syndrome and recurrent pregnancy loss. *Ann NY Acad Sci* 1994; 731: 242–245.
57. Bertolaccini ML, et al. 'Non-criteria' aPL tests: report of a task force and preconference workshop at the 13th International Congress on Antiphospholipid Antibodies, Galveston, TX, USA, April 2010. *Lupus* 2011; 20: 191–205.
58. Bertolaccini ML, et al. Revisiting antiphospholipid antibodies: from targeting phospholipids to phospholipid binding proteins. *Clin Lab* 2004; 50: 653–665.
59. Nayfe R, et al. Seronegative antiphospholipid syndrome. *Rheumatology* 2013; 52: 1358–1367.
60. Rai R, et al. Randomised controlled trial of aspirin and aspirin plus heparin in pregnant women with recurrent miscarriage associated with phospholipid antibodies (or antiphospholipid antibodies). *Br Med J* 1997; 314: 253–257.
61. Goel N, et al. The role of aspirin versus aspirin and heparin in cases of recurrent abortions with raised anticardiolipin antibodies. *Med Sci Monit* 2006; 12: CR132–CR136.
62. Farquharson RG, et al. Antiphospholipid syndrome in pregnancy: a randomized, controlled trial of treatment. *Obstet Gynecol* 2002; 100: 408–413.
63. Rai RS, et al. High prospective fetal loss rate in untreated pregnancies of women with recurrent miscarriage and antiphospholipid antibodies. *Hum Reprod* 1995; 10: 3301–3304.
64. Mak A, et al. Combination of heparin and aspirin is superior to aspirin alone in enhancing live births in patients with recurrent pregnancy loss and positive anti-phospholipid antibodies: a meta-analysis of randomized controlled trials and meta-regression. *Rheumatology* 2010; 49: 281–288.
65. Bates SM, et al. VTE, thrombophilia, antithrombotic therapy, and pregnancy: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. *Chest* 2012; 141: e691S–e736S.