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 β2 glycoprotein I (anti-β2GPI) 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. 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 anti-β2GPI, or low positive positive aCL or anti-β2GPI from APS laboratory diagnostic criteria may result in missing the diagnosis in a sizable 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

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 commonest 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 >99th centile); and/or anti-β2-glycoprotein-1 (anti-β2GPI) (IgG and/or IgM) antibodies >99th 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...
Obstetric antiphospholipid syndrome

Clinical manifestations of obstetric APS

- One or more unexplained deaths of a morphologically normal fetus at or beyond the 10th week of gestation
- One or more pre-term births of a morphologically normal neonate before the 34th week of gestation because of:
  - (i) eclampsia or severe pre-eclampsia or
  - (ii) recognised features of placental insufficiency
- 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

Laboratory criteria

- LA present in plasma, on two or more occasions at least 12 weeks apart
- aCL of immunoglobulin (Ig)G and/or IgM isotype in serum or plasma, present in medium or high titre (i.e. >40GPL units or MPL units, or > the 99th centile), on two or more occasions, at least 12 weeks apart
- aβ2GPI of IgG and/or IgM isotype in serum or plasma (titre > the 99th centile), present on two or more occasions at least 12 weeks apart

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; aβ2GPI: antiβ2glycoprotein-I antibodies.

Search criteria

A literature search was carried out on PubMed for publications in non-criteria clinical and/or laboratory manifestations should be included to address the question of whether patients who exhibit 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.

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 αβ2GPI (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 syncytiotrophoblast, trophoblasts express cell membrane anionic phospholipids that can bind β2GPI. aPL which are β2GPI-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 β2GPI/anti-β2GPI 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 (EUROAPS) 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-
event, 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 13th 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 aβ2GPI positivity during the normal child bearing years as these studies (38–40) were generally undertaken prior to the inclusion of aβ2GPI in the international consensus laboratory criteria (1, 2). This makes it difficult to distinguish whether the presence of low positive aβ2GPI 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 aβ2GPI should be dropped from the laboratory criteria for APS and only IgG aβ2GPI 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 aβ2 GPI) is associated with a significantly higher rate of pregnancy loss in obstetric APS (46), a single positive aPL test, more often aCL or aβ2GPI 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 aβ2GPI) and 31% (34/109) aCL alone (45). In the NOH-APS observational study, positivity for IgM aCL was an independent risk factor for placenta-mediated complications. Positive results for LA, IgG aβ2GPI 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 aβ2GPI) in diagnosing obstetric APS.

<table>
<thead>
<tr>
<th>Reference and year</th>
<th>Number of patients with obstetric APS</th>
<th>Laboratory criteria used to diagnose obstetric APS</th>
<th>Repeat testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retrospective observational cohort study (8) 2010</td>
<td>176/693 (25%)</td>
<td>aCL levels &gt;95th centile healthy adults aβ2GPI were not tested</td>
<td>Yes (100%) 6 or more weeks apart</td>
</tr>
<tr>
<td>Retrospective Cohort study (13) 2012</td>
<td>As per ICS criteria 23/25 (92%) Low aPL 27/32 (84%) had only APS-like obstetrical events</td>
<td>1. ICS laboratory criteria for aCL and aβ2GPI 2. Low titre IgG/IgM aβ2GPI and/or IgG/IgM aCL (&gt;90th&lt;99th percentile)</td>
<td>Yes (100%) 12 weeks apart</td>
</tr>
<tr>
<td>Prospective registry (14) 2012</td>
<td>194/226 (85.8%) OAPS 32/226 (14.6%) OMAPS.</td>
<td>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 aβ2GPI (levels&lt;99 centile)</td>
<td>Yes (89%) 12 weeks apart or detected once</td>
</tr>
<tr>
<td>Retrospective audit (10) 2013</td>
<td>40/145 (28%)</td>
<td>1. ICS laboratory criteria for aCL and aβ2GPI 2. Low titre IgG/IgM aCL I and/or IgG/IgM aβ2GPI (&gt;95th&lt;99th percentile) Reference ranges for aCL and aβ2GPI were obtained from 240 normal healthy volunteers</td>
<td>Yes (100%) 12 weeks apart</td>
</tr>
</tbody>
</table>

APS: Antiphospholipid syndrome; aCL: anticardiolipin antibodies; aβ2GPI: anti-β2glycoprotein-1 antibodies; ICS: international consensus; OAPS: Obstetric antiphospholipid syndrome; OMAPS: obstetric morbidity associated to antiphospholipid antibody syndrome; aPS: antiphosphatydilserine; 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 IgG aβ2GPI 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 aβ2GPI 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 aβ2GPI 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 aβ2GPI 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 aβ2GPI 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 aβ2GPI 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 aβ2GPI in normal populations. Reference ranges for aCL and aβ2GPI 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 aβ2GPI 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 aβ2GPI levels between the 90th and 99th centile (13). This group was compared with those women who had aCL and aβ2GPI 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 aβ2GPI 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 β2GPI, 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 an 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 (<99 th 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
References


