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Review

Obstetric antiphospholipid syndrome: A recent classification for an old defined disorder

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ABSTRACT

Obstetric antiphospholipid syndrome (APS) is now being recognized as a distinct entity from vascular APS. Pregnancy morbidity includes >3 consecutive and spontaneous early miscarriages before 10 weeks of gestation; at least one unexplained fetal death after the 10th week of gestation of a morphologically normal fetus; a premature birth before the 34th week of gestation of a normal neonate due to eclampsia or severe pre-eclampsia or placental insufficiency. It is not well understood how antiphospholipid antibodies (aPLs), beyond their diagnostic and prognostic role, contribute to pregnancy manifestations. Indeed aPL-mediated thrombotic events cannot explain the obstetric manifestations and additional pathogenic mechanisms, such as a placental aPL mediated complement activation and a direct effect of aPLs on placental development, have been reported. Still debated is the possible association between aPLs and infertility and the effect of maternal autoantibodies on non-vascular manifestations in the babies. Combination of low dose aspirin and unfractionated or low molecular weight heparin is the effective treatment in most of the cases. However, pregnancy complications, in spite of this therapy, can occur in up to 20% of the patients. Novel alternative therapies able to abrogate the aPL pathogenic action either by interfering with aPL binding at the placental level or by inhibiting the aPL-mediated detrimental effect are under active investigation.

Contents

1. Introduction

Antiphospholipid syndrome (APS) was firstly described as a disorder in 1983. It was defined as an autoimmune disease and/or a prothrombotic condition characterized by the presence of circulating antiphospholipid antibodies (aPLs), as well as peripheral thrombosis (e.g., deep vein thrombosis), repeated miscarriage, and, occasionally,

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thrombocytopenia [1]. APS can be isolated (primary) or associated with other systemic autoimmune diseases (secondary), mainly systemic lupus erythematosus (SLE; around 30% of patients), which affects 1 to 20 in every 100,000 women, depending on ethnic origin [2,3]. The population prevalence of primary APS is uncertain: it is estimated to affect about 0.5% of the population [2,3]. The male:female ratio is 1:3.5 for primary disease and 1:7 for secondary APS [3–6]. The mean age at diagnosis is 35 years, and APS rarely occurs in children (Table 1).

Even though the syndrome was described as a unique disorder, the distinction between obstetric APS and vascular APS has been well established during the last ten years given the observation that i) patients can display vascular thrombosis with no pregnancy complications or, alternatively, obstetric manifestations alone [7] and ii) the coexistence of both thrombosis and miscarriage only affects about 2.5–5% of APS pregnancies [8]. This might occur because the clinical and biological characteristics of the vascular involvement are different from those associated with the obstetrical problems. Indeed, the thrombotic phenomena do not have the sole responsibility for the obstetrical complications, for which involvement of additional mechanisms has been reported [9–11]. Nevertheless, the Nimes Obstetricians and Hematologist Antiphospholipid Syndrome (NOH-APS) observational study compared the incidence of thrombotic events in 517 women with purely obstetrical APS to 796 seronegative women with a history of pregnancy loss. The annual rate of thrombotic events (deep vein thrombosis, pulmonary embolism, superficial vein thrombosis, cerebrovascular events) was found to be higher in the aPL-positive group than in the other one [12]. Therefore, though their frequency remains usually low, thrombotic events occur more frequently in patients with obstetric APS compared to aPL-negative patients with poor obstetric history [13].

To date, the true boundaries of the syndrome remain undefined. It is questioned: i) whether APS is primarily a coagulation disorder induced by antibodies or if this is a more complex autoimmune disease in which the different manifestations are caused by the different antibodies; ii) whether patients presenting only with significantly elevated aPL levels and no clinical features or with aPL-associated "non-thrombotic features" should be considered at risk of future thrombosis or pregnancy complications. We are not able to answer these questions, even though the development of criteria to identify patients with APS has been important for more rigorous clinical and laboratory studies to be performed. In the present review we will focus on the obstetric APS and on the results obtained through basic research and clinical studies that we performed over the last few years. We strongly believe that these contributions witness our commitment to advance our understanding of obstetric APS and make a step further in the knowledge of the topic.

Table 1
Antiphospholipid antibody syndrome (APS) clinical criteria and laboratory criteria. Modified from Ref. [6].

Clinical criteria

1. Vascular thrombosis
One or more clinical episodes of arterial, venous, or small vessel thrombosis, in any tissue or organ. Thrombosis must be confirmed by objective validated criteria (i.e. unequivocal findings of appropriate imaging studies or histopathology). For histopathologic confirmation, thrombosis should be present without significant evidence of inflammation in the vessel wall.
2. Pregnancy morbidity
   (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 preeclampsia, or (ii) recognized features of placental insufficiency, or
   (c) Three or more unexplained consecutive spontaneous abortions before the 10th week of gestation, with maternal anatomic or hormonal abnormalities and paternal and maternal chromosomal causes excluded.

Laboratory criteria

1. Lupus anticoagulant (LA) present in plasma, on two or more occasions at least 12 weeks apart.
2. Anticardiolipin (aCL) antibody of IgG and/or IgM isotype in serum or plasma, present in medium or high titer (i.e. >40 GPL or MPL, or >the 99th percentile), on two or more occasions, at least 12 weeks apart, measured by a standardized ELISA.
3. Anti-β2 glycoprotein-I antibody of IgG and/or IgM isotype in serum or plasma (in titer >the 99th percentile), present on two or more occasions, at least 12 weeks apart, measured by a standardized ELISA.

International classification criteria for this syndrome were proposed in 1998 in Sapporo (Japan) and updated in 2006 in Sydney (Australia). At least one clinical manifestation together with positive laboratory tests is required to fulfill the classification criteria.
Beyond classical criteria "non-criteria" clinical and laboratory manifestations should be mentioned. Non-criteria clinical findings include: heart valve disease; livedo reticularis; thrombocytopenia; nephropathy; neurological manifestations [5] Non-criteria laboratory findings include: IgG or IgM aCL or anti-β2GPI levels in the range of 20 to 39 GPL or MPL units; IgA aCL and IgA anti-β2GPI; anti-phosphatidylserine (aPS) and anti-phosphatidylethanolamine antibodies (aPEs); anti-prothrombin antibodies (aPTs) and antibodies to the phosphatidylserine–prothrombin complex (aPS/PT). It is not known whether the above mentioned conditions may "predate" the development of APS and may be regarded as "pre-APS" manifestations. Until such information has been well established they cannot be included in the diagnostic criteria because of potential loss of specificity in recognizing APS, which might lead to unwarranted treatments.

2. Dual function of antiphospholipid antibodies (aPLs) as diagnostic criteria and risk factors

Laboratory testing for aPLs represents a crucial step in defining patients affected by APS since none of the clinical criteria associated with APS are specific to this condition. The detection of aPLs requires an organized approach because of a considerable intra-assay and inter-laboratory variation in the results, especially when samples contain low antibody titers, and when, in the case of LA, anticoagulant therapy may interfere with the results [14].

LA assay is a functional assay based on a combination of different clotting tests. It detects the presence of immunoglobulins which in vivo are associated with thrombosis and, paradoxically, in vitro are able to prolong PL-dependent coagulation tests. In order to improve test performance, the recently updated guidelines recommend that: LA test should be performed only in selected patients with a significant suspicion of having APS or with an unexplained prolongation of activated partial thromboplastin time (aPTT); it should be performed before the start of any anticoagulant drug or a sufficient period after its discontinuation; only two screening tests should be used, the dilute Russell's viper venom time test (dRVTT) as first choice, while the second test should be a highly sensitive aPTT performed with silica as an activator and with a low content of PL [14].

The aCL and anti-β2GPI are detected by ELISA. aCL ELISA mainly detects antibodies against CL-bound β2GPI. The anti-β2GPI ELISA represents the only test which identifies aPLs directed against the β2GPI, generally regarded as the major target antigen for aPLs in APS patients [15,16]. During the years, the methodological limitations of aPL ELISAs have been so debated that, in a recent editorial, aPLs were strongly criticized as useful criteria for the diagnosis of APS [17]. To our opinion, restarting an old dispute might result to redundancy before conclusive novel evidence can support the idea that laboratory criteria for APS need to be changed: 30 years of literature on aPLs cannot be considered *en bloc* at the same level. We agree with Pierangeli et al., when they identify three different periods within the history of APS [18]. The first one was an "observational" period, during the years 1953 to 1983; the second was a period of "exponential growth in interest" (1983 to 1995); and, finally, the current one starting from 1995 to the present. This latter is a period of "consolidation and refinement" in the laboratory procedures used to detect aPLs and in understanding the syndrome. To date, there is no doubt that aCL and anti-β2GPI testing is crucial for the diagnosis of APS. After all, variation in the results in both aCL and anti-β2GPI testing remains a concern which extends to many other autoimmune disease-related tests (e.g., anti-dsDNA or anti-citrulline) and limits their clinical utility. We acknowledge that, in the early years, the studies in the literature showed several technological biases (lack of an international calibrator, different types of plate etc.), however the emergence of new platforms and detection technologies using semi-

or fully-automated analyzers is now contributing to the improvement of the results in terms of standardization and reproducibility [19–22].

In the last few years a large number of studies attempted to quantify the risk for clinical complications in patients with aPLs: a common challenge for researchers is that of stratifying patients with similar clinical manifestations but different patterns and combinations of positive aPLs [23,24], in particular those with: 1) low versus high titers of antibodies; 2) single or multiple isotype of aCL and anti-β2GPI; 3) single, double or triple aPL positivity. There is evidence that high titers and the IgG isotype of aCL and anti-β2GPI correlate better with aPL-related clinical events compared to low titers and the IgM isotype [25,26]. The stratification of the risk for thrombotic events has been well studied; conversely studies evaluating the obstetric risk still remain scant. Ruffatti et al. reported that high titers and triple positivity for aPLs and/or a history of thromboembolism can predict the occurrence of negative events in subsequent pregnancies, even when treatment is well conducted [27,28]. More recently, Bramham et al. confirmed that women with thrombotic APS have higher rates of pregnancy complications than those with obstetric APS alone [29].

3. The clinical picture of obstetric APS

Pregnancy morbidity in APS encloses fetal and maternal complications. In particular:

- ≥3 consecutive and spontaneous early miscarriages before 10 weeks of gestation
- at least one unexplained fetal death after the 10th week of gestation of a morphologically normal fetus
- a premature birth before the 34th week of gestation of a normal neonate due to eclampsia or severe pre-eclampsia or placental insufficiency [3,30].

The most frequent fetal complication in APS is recurrent pregnancy loss (RPL). It is difficult to establish the frequency of APS in patients with RPL because of the high variability in the definition of RPL (number of the pregnancy losses required, 2 or 3). However it is estimated that between 7% and 25% of RPL are due to the presence of aPLs (before 10 weeks of pregnancy) [31–36]. Further fetal complications in APS patients include prematurity, intrauterine growth restriction due to placental insufficiency and stillbirth. The Euro-Phospholipid Project study, which analyzed the clinical characteristics of 1000 patients with APS during a 5 year follow-up, estimated that these events complicate 28%, 11% and 7% of APS pregnancies respectively [36].

The most common maternal manifestation of APS is preeclampsia, followed by eclampsia and abruptio placentae [37,38]. Preeclampsia generally affects 2–8% of pregnancies [39]. Conflicting results have been reported about the real frequency of aPLs in patients with preeclampsia, possibly due to differences in the inclusion criteria. A meta-analysis reported an odds ratio for the association of aCL with preeclampsia of 2.86 [95% CI, 1.37–5.98] and an odds ratio for that with severe preeclampsia of 11.15 [95% CI, 1.37–5.98]. Despite this significant association, the authors concluded that there is insufficient evidence to use aCL as predictors of PE [40]. Considering patients positive for aPLs, a cross-sectional study conducted in Florida on 141,286 women who delivered in 2001 showed that high titers of aPLs ($n = 88$) increase the risk of PE or eclampsia with an odds ratio of 2.93 [41].

It is not yet understood how aPLs contribute to pregnancy complications in APS patients. Intraplacental thrombosis was initially suggested as the main pathogenic mechanism underlying the poor obstetric outcome [42,43]. However, the failure of subsequent studies in showing intravascular/intervillous blood clots in the majority of APS placentas [44–46] led to hypothesizing the occurrence of additional pathogenic events. In particular it has been suggested that aPLs might induce a direct negative effect on the human placentation [44,46], a process which requires both the invasion of trophoblast cells into maternal tissues and the formation of new vessels in the decidualized endometrium [47]. During invasion, trophoblast cells migrate through the uterine endometrium, the inner third of myometrium and the uterine vasculature [48]. To this end trophoblasts secrete metalloproteinases (MMPs), specific proteolytic enzymes capable of degrading all components of the extracellular matrix [48]. On the other hand, the formation of new vessels in the endometrium ensures the development of the feto-maternal vasculature for the adequate delivery of nutrients to the developing embryo [49]. In particular decidualizing endometrial cells produce critical angiogenic molecules, such as Vascular Endothelial Growth Factor (VEGF) able to promote the angiogenic differentiation of endometrial endothelial cells [50].

Based on the evidence of the β2GPI placental tropism, we previously observed that polyclonal IgG antibodies from APS patients and human IgM monoclonal antibodies with anti-β2GPI activity can adhere both to human trophoblast cells and to endometrial endothelial cells (HEECs) in vitro [51–53]. Subsequently, we aimed at investigating whether, after binding placental tissue, aPLs might induce a functional damage which could correlate with the poor obstetric outcome. Accordingly we observed that aPLs are able to:

(i) inhibit syncytiotrophoblast differentiation, as shown by the reduced secretion of human chorionic gonadotrophin (hCG) [51,52];
(ii) impair trophoblast invasiveness in an in vitro Matrigel assay. This effect is well correlated with a significant inhibition of expression/activity of MMPs [51,52];
(iii) affect the trophoblast expression of integrins and cadherins. These represent adhesion molecules, whose expression is regulated during the process of trophoblast adhesion and invasion into maternal tissues. In particular we found that aPLs decrease alpha 1 integrin and VE-cadherin and up-regulate alpha 5 integrin and E-cadherin [53].
(iv) block endometrial angiogenesis both in vitro and in vivo, by inhibiting the HEEC tube formation and the production of specific factors up-regulated during angiogenesis, such as VEGF [54]. Studies on the involvement of β2GPI in the angiogenesis showed that, in contrast to the previously reported anti-angiogenic properties of β2GPI [55], a cleaved form of β2GPI is able to block the activity of angiostatin, a well known inhibitor of angiogenesis [56]. We only investigated the effect of anti-β2GPI antibodies on endometrial angiogenesis: whether the observed inhibitory effect is due to an imbalance between the intact and the cleaved form of β2GPI still remains to be explored.

Taken together our findings provided novel important evidence whereby aPLs, by disrupting trophoblast invasion and endometrial angiogenesis, might contribute to a defective placentation and, in turn, affect the successful beginning of pregnancy.

A further pathogenic mechanism proposed for the APS obstetric morbidity is inflammation [57,58]. Although there are few retrospective reports on inflammatory signs in the placentas of APS women, the issue is still debated. Evidence from experimental animal models confirmed the ability of large amounts of human aPL IgG, passively infused after implantation, to induce fetal resorption and growth retardation through strong placental IgG and complement deposition, neutrophil infiltration and local TNF-α secretion [59,60]. Further in vivo studies obtained by injection of small amounts of human aPL IgG before implantation [61] still showed the ability of the antibodies to induce fetal loss and growth retardation but without any sign of acute local inflammatory events and complement deposition [61]. A possible explanation for these discrepancies may be related to the different experimental designs. Moreover, the finding that histological examination of the human term placenta does not support a strong inflammatory signature may be due to aPL-mediated events taking place at the beginning of the pregnancy that can display just the resulting damage rather than the acute process at the time of the histological examination. Taken together, these findings suggest that, regardless of the effects of aPLs, mechanisms underlying

pregnancy complications in APS patients are heterogeneous, complex, and not fully understood. Nevertheless, the ability of aPLs to directly target both the fetal side (invading trophoblast) and the maternal one (decidua and endometrial endothelial cells) of the human placenta and to induce a negative effect on placentation not necessarily related to prothrombotic or inflammatory events is nowadays well established.

4. Non-criteria aPLs and pregnancy complications

In addition to classical aPLs, increasing evidence demonstrates the presence of several autoantibodies shown to be directed to negatively charged PL other than cardiolipin, including phosphatidylethanolamine (aPE), to other PL-binding proteins (i.e. prothrombin (aPT) and/or phosphatidylserine–prothrombin complex, aPT/PS) or to interfere with the anticoagulant activity of annexin 5. All together these autoantibodies have been proposed to be relevant in obstetric APS, but controversial conclusions have been reported [62–66].

aPEs are directed against phosphatidylethanolamine, one of the main lipid components of the microbial membranes where it seems to work as a molecule involved in the folding of other membrane proteins [63]. Its location in the inner leaflet of biological membranes suggests that a primary event in APS patients possibly exposes PE in the outer part of the membrane, making it possible for the aPLs to bind. During the last two decades several studies have documented the association of aPEs with pregnancy morbidity [63–66], starting since 1996, when Yetman and Kutteh reported a positivity for non-criteria aPLs, including aPEs in 10.1% of women with RPL [67]. Further studies reported an increased incidence of aPEs in patients with early pregnancy losses and mid-to-late pregnancy losses in comparison to controls [68,69]. In 2007, aPE was detected with an increased frequency (67.5%) also in infertile women with previous ≥3 recurrent implantation failures, and in 70% of the cases aPE positivity was found in the absence of other aPLs [70]. However, aPE was not shown to be an independent risk factor for further miscarriage in patients with RPL [71]. Finally according to a recent report, the combinations of IgG aPEs plus IgG aCL, or IgG aPEs plus LA measurements can be useful predictors of severe pregnancy-induced hypertension, with a 30.8% sensitivity and a 99.2% specificity [71].

Anti-prothrombin antibodies are directed against prothrombin (PT), a vitamin-K-dependent single-chain glycoprotein involved in the coagulation processes. aPTs are low affinity antibodies recognized more efficiently when the PT is bound to phosphatidylserine (PS) coated on ELISA plates via calcium ions. ELISAs used to detect aPT/PS identify an autoantibody population partially different from the assay using PT as the only antigen [72]. It has been suggested that aPTs might exert thrombogenic effect by increasing the affinity of PT for negatively charged PL, thereby competing with clotting factors for the available catalytic PL surface. However no clinical association between isolated aPTs and the risk of thrombotic events was found in a systematic review [73]. On the other hand the aPS/PT positivity has been strongly associated with aPL-associated manifestations, mainly thrombotic events. Less clarified remains the association of aPTs and aPL-related pregnancy morbidity. Shoenfeld et al. analyzed a group of 109 patients with RPL and 120 healthy volunteers. They found that in the RPL group, aPTs were more prevalent than in controls with an odds ratio (OR) of 5.4 [74]. These observations were in line with an earlier study which demonstrated higher levels of aPTs in RPL patients [75] and further studies reporting a high prevalence of aPTs in women with early pregnancy loss either associated [76] or not [77] with APS. Also Sater et al. confirmed such observations, even though they concluded that the association of aPTs with RPL remains controversial because, in their study, it disappeared after controlling for a number of covariates [78]. Beyond RPL, Marozio et al. investigated the prevalence of aPTs in 187 patients negative for classical aPLs and with previous "vascular adverse outcomes of late pregnancy" including severe PE and/or Hemolysis, Elevated Liver Enzyme and Low Platelets (HELLP) syndrome and/or placental abruption and/or intrauterine fetal death. They reported a significantly 10-fold higher prevalence of aPT IgG in cases than in controls (OR, 95% CI: 10.92, 4.52–26.38). Furthermore, a subanalysis, according to each of the four previous pregnancy complications, demonstrated that the prevalence of aPTs was higher in the four groups of cases than controls, reaching the strongest statistical association with intrauterine fetal death (OR, 95% CI: 10.80, 2.35–49.67; $p < 0.001$) [79].

Anti-annexin 5 antibodies are directed against annexin 5, a placental anticoagulant protein highly expressed on the apical surfaces of syncytiotrophoblast, where it plays a thrombomodulatory role and contributes to the fluidity of the maternal circulation through the intervillous space. Anti-annexin 5 IgG antibodies have been reported with higher frequency in 518 women with unexplained fetal loss compared with the same number of women with explained fetal loss, and women with no previous obstetrical complications, suggesting that these antibodies may represent a risk factor for fetal loss [80]. Matsubayashi et al. showed that 5.5% of women with RPL are anti-annexin 5 antibodies positive, compared with 1.1% of normal non-pregnant or pregnant healthy women [81]. In line with these results, Sater et al. reported a significant elevation in anti-annexin 5 IgM and IgG in unexplained RPL patients compared to controls [82]. Conversely a large prospective study failed to demonstrate an association between anti-annexin 5 antibodies detected at the beginning of pregnancy and the prediction of miscarriage, thus failing to support the usefulness of these antibodies in the evaluation of obstetrical risk of miscarriage [83]. When analyzing the association with other aPLs, a significantly increased prevalence of anti-annexin 5 in aPL-positive women with RPL has been observed compared with aPL negative women who develop the same obstetrical complication or with healthy parous women (35%, 19% and 15%, respectively) [84]. This lead to the suggestion that anti-annexin 5 antibodies do not represent an independent risk factor for RPL and that the association between anti-annexin 5 antibodies and RPL might remain controversial in the general population, while in APS patients the aPL-mediated disruption of the annexin 5 crystal shield could represent a mechanism leading to pregnancy loss and thrombosis [85]. Indeed, aPLs have been shown to reduce the quantity of annexin 5 on cultured placental trophoblasts and to accelerate the coagulation of plasma that is exposed to these cells [85,86]. Accordingly Rand et al. [87] reported that women with obstetric APS show an increased annexin 5 resistance compared with controls and patients with isolated aPLs and that such resistance is well correlated with levels of anti-β2GPI IgG hence hypothesizing that annexin 5 resistance is a mechanism for pregnancy losses associated with β2GPI-dependent aPLs.

5. Is there a role for aPLs in patients with unexplained sterility?

Given the ability of aPLs to affect implantation, placentation and early embryonic development, authors have recently suggested the possibility that aPLs may be responsible also for sterility [88]. Sauer et al. evaluating 1325 women with unexplained sterility and 676 women with recurrent implantation failure reported a significantly higher positivity for aPLs when compared with fertile negative controls (8–9% vs 1.5%; $p < 0.0001$) [89]. Beyond the well known mechanisms by which aPLs impact the frequency of pregnancy complications, in vitro and in vivo studies have shown the ability of aPLs to exert a direct negative effect on uterine endothelium and preimplantation embryos [90–92]. Since angiogenesis is necessary for uterine receptivity and since aPLs inhibit endometrial angiogenesis [54,93], the effect on the uterus could be mediated through the process of angiogenesis. Furthermore early studies, using high doses of aCL but with anti-β2GPI independent activity, reported a direct detrimental effect of aPLs on the morphology of preimplantation embryos [91]. Thus it could be suggested that, depending on the cellular target (endometrial endothelial cells, trophoblast cells and preimplantation embryos), aPLs might

contribute to different clinical manifestations. In a schematic way it could be suggested that:

- the inhibitory effect of aPLs on endometrial endothelial cell angiogenesis and on trophoblast differentiation, proliferation and invasion would lead to defective placentation clinically resulting in RPL or recurrent implantation failure;
- a possible direct toxic effect on the preimplantation embryo would result in unexplained sterility or recurrent implantation failure.

However, because of poorly designed studies, there is still a lack of evidence for aPL predictive value on implantation outcome [94–96]. The exact role of aPLs in determining sterility remains unknown, and until high quality studies investigating the association of aPLs with sterility are performed, testing latent aPLs in this group of patients remains a choice based on the isolated expert advice.

6. Babies born to mothers with APS

The European aPL Forum has recently published the results of a multicenter prospective registry including a cohort of babies born to mothers with APS in seven European obstetric centers.

The registry analyzed the immunological status of the babies, their neonatal outcome, and the long-term follow-up, which started from birth up to the age of 5 [97]. In line with previous results, a high rate of prematurity (<37 weeks; 16%) and of small for gestational age neonates (17%) despite maternal treatment has been found [98,99]. Furthermore no neonatal lupus, SLE, or thrombotic events have been recorded during the 5-year follow-up. For this reason it was initially hypothesized that most of the potential pathogenic aPLs were absorbed at the placental level (where β2GPI is highly expressed) and not transferred to the fetus. However, transplacental transfer of aPLs occurred in these pregnancies: in particular at birth LA was present in 4%, aCL IgG in 16%, anti-β2GPI IgG/M in 15%–3% and triple positivity in 3% of cases. After 6 months, aCL IgG antibodies were still present in 20% of children and anti-β2GPI IgG in 33%. aPLs persisted in 10% of children, whereas de novo anti-β2GPI IgG appeared in 16%. Still open is the question whether or not the apparently most pathogenic anti-β2GPI IgG subpopulations – for example the antibodies reacting with Domain I epitope – can be transferred as those directed against other parts of the molecule [100].

With respect to the long-term follow-up, two previous retrospective reports [98,99] showed learning disabilities in 15–20% of children born to mothers with APS. In line with such results, the European registry reported that the prevalence of neurodevelopmental disabilities was twofold higher than the general population (1%). These abnormalities included hyperactive behavior, feeding disorders, language delay, and autism. The presence of autism was recently found to be more prominent in children born prematurely and/or weighting less than 2000 g [101]. Because of the high rate of prematurity and small for gestational age neonates in the APS group, this could constitute an additional factor of neurodevelopmental abnormalities in APS-exposed children (Table 2). The influence of aPLs upon children's neurological development is not yet understood. Nervous system involvement, possibly due to thrombotic processes in the vessels of the brain, is a prominent feature of APS in adults. Several neurological manifestations, which may even precede the diagnosis of full blown APS, have been described including cognitive dysfunction, memory alterations, mood disorders, anxiety, impulse control disorders and psychosis [102–106]. Children with APS do not show thrombotic events [107], therefore additional mechanisms of aPL-mediated neurological damage, such as activation of endothelial cells and disturbance of blood–brain barrier, should be suggested in these patients. Although the European registry's results are preliminary and should be extended and confirmed, the reported findings might justify a systematic psychomotor and cognitive follow-up in children born to APS patients.

7. Treatment of obstetric APS

In obstetric APS patients, several strategies have been proposed to improve the pregnancy outcome, including combinations of aspirin and unfractionated (UFH) or low molecular weight (LMWH) heparin [108–110]. However, there is no clear evidence whether UFH and LMWH have comparable efficacy. Treatment with heparin is mainly based on the initial assumption that thrombotic events play the major role [109,110]. Because of the finding that thrombosis cannot explain all the aPL-mediated complications, the anti-inflammatory activity of heparin has been then advocated [111], but the lack of clear evidence for an inflammatory mechanism at the placental level was not able to support such explanation. The presence of alternative mechanisms of placental damage in APS and the success of treatment with heparin on the pregnancy outcome led to hypothesize additional mechanisms of action for the drug [111,112]. We previously demonstrated that LMWH is able to prevent the binding of aPLs to trophoblast cells and to restore in vitro placental invasiveness and differentiation [112]. Accordingly, it was shown that the primary heparin-binding site of β2GPI is the positively charged site located within the D5 of the protein, where also the PL-binding site was demonstrated [113]. Then heparin seems to prevent the binding of β2GPI to negatively charged PL, which in turn prevents the deposition of the anti-β2GPI in tissues. In subsequent studies we also observed that heparin is able to block the aPL-mediated inhibition of HEEC angiogenic differentiation [114]. We, therefore, could suggest that heparin, by interfering with the aPL binding, is able to prevent the aPL pathogenic action not only on the fetal side of the placenta (trophoblast cells), but also on the maternal one (endometrial endothelial cells).

The likelihood of a good pregnancy outcome in women with APS is around 75–80% under correct management. Unfortunately, there is a significant proportion of women, about 22%, which do not respond to the standard treatment and still suffer from miscarriages and adverse pregnancy events [115,116]. The role of glucocorticoids remains still worthy of further assessment. The addition of prednisolone (at doses of 40–60 mg) to aspirin alone or associated to heparin during gestation has shown no clear benefits in APS pregnant women and has been associated to important side effects, such as preterm delivery because of premature rupture of membranes or preeclampsia [117–119]. By contrast, a recent study by Brahman et al. seems to suggest that the addition of low-dose prednisolone (10 mg) from the time of positive pregnancy test up to 14 weeks of gestation may be effective in increasing live birth rate [120].

The addition of intravenous immunoglobulin (IVIg) has not been shown to be superior to heparin and aspirin in unselected patients. This was recently confirmed in a multicenter clinical trial that tested the effect of IVIg compared with LMWH plus low-dose aspirin for the treatment of women with APS and recurrent miscarriage. The rate of live births was 72.5% in the group treated with heparin plus aspirin compared with 39.5% in the immunoglobulins group [121]. Noteworthy, another study reported the possible efficacy of IVIg in obstetric APS patients selected for poor prognosis or autoimmune phenomena [122]. A possible explanation for the high proportion of refractory APS might be that not all the mechanisms underlying aPL-mediated pregnancy complications have been clarified. Novel alternative therapies are urgently needed [123]. Given the evidence that β2GPI and the related autoantibodies play a central role in aPL-mediated obstetric manifestations, new molecules able to interfere with β2GPI expression at the placental level have been recently investigated, in particular, a synthetic peptide, TIFI, sharing structural similarity with the PL-binding site of β2GPI. Through this similarity, TIFI can compete with the β2GPI PL-binding site and displace the molecule from the cell surface, ultimately inhibiting aPL binding to the target tissues [124]. Accordingly, this peptide was previously found to prevent aPL-mediated thrombosis in vivo and inhibit the in vitro binding of β2GPI to human endothelial cells and murine monocytes [124]. In line with such observations, we

Table 2
Children's general characteristics, neurodevelopment and follow-up during 5 years.
Modified from Ref. [97].

	At birth (n = 130)	3 months (n = 110)	9 months (n = 105)	24 months (n = 64)	5 years (n = 27)
Weight (kg)	3 ± 0.5	5.7 ± 1.1			
Weight (<2 SD)	–	3 (3%)	4 (4%)	0	0
Height (cm)	48 ± 3	58 ± (21)	71 ± 5	84 ± 7	111 ± 10
Height (<2 SD)	–	9 (9%)	9 (9%)	0	0
Cranial perimeter (cm)	34 ± 2	40 ± 2	45 ± 2	48 ± 2	50 ± 2
Cranial perimeter (<2 SD)	–	0	2 (2%)	0	–
Infections	5 (4%)	6 (5%)	10 (10%)	11 (17%)	–
Atopy	–	8 (7%)	8 (7%)	7 (11%)	1 (4%)
Lupus	0	0	0	0	0
Thrombosis	0	0	0	0	0
Neurodevelopmental abnormality	–	1 (1%)	1 (1%)	3 (5%)	2 (7%)
Neurodevelopmental abnormality description	–	Axial hypotony	Axial hypotony, psychomotor delay	Autism, hyperactive behavior, feeding disorders, language delay, growth failure	Autism, hyperactive behavior

Each column represents the number of evaluated children at the check point.

recently reported the ability of TIFI to inhibit the β2GPI binding to trophoblast cells in vitro, and, when passively infused in naïve pregnant mice, to prevent the aPL-mediated fetal loss [125]. In subsequent studies, we showed that TIFI is able to block the aPL-mediated inhibition of HEEC angiogenesis in vitro, providing an additional mechanism whereby this peptide prevents the aPL effect at the placental level [126]. As a whole, these results show how TIFI, by affecting the β2GPI placental expression, can inhibit the binding of β2GPI-dependent aPLs and, in turn, the trophoblast and endometrial endothelial cell aPL-mediated damage. At the same time, these results suggested a safer and more specific therapeutical approach able to abrogate the aPL pathogenic effects. Indeed, the observation that mice lacking β2GPI show a compromised early pregnancy suggested that functional β2GPI is necessary for optimal implantation and placental morphogenesis [127]. This has led researchers to conceive novel and promising biological therapies useful for refractory APS patients and based on the use of immunomodulatory drugs [128] or a monoclonal antibody which are able to prevent the aPL-mediated activation of the complement and the pro-coagulant and proabortive effects, without interfering with the expression of the placental β2GPI [129,130].

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Competing interests

The authors declare that they have no conflicts of interest in the research.

Take-home messages

- Obstetric APS is now being recognized as a distinct entity from vascular APS.
- aPLs are diagnostic, predictive and pathogenic autoantibodies for both the obstetric and the vascular APS.
- aPL-mediated thrombotic events cannot explain the obstetric manifestations and additional pathogenic mechanisms have been suggested such as a placental complement-dependent inflammation and a direct effect of aPLs on placental development.
- Still debated is the possible association between aPLs and infertility and the effect of maternal autoantibodies on non-vascular manifestations in the babies.
- Combination of low dose aspirin and unfractionated or low molecular weight heparin is the effective treatment in most of the cases. However, pregnancy complications, in spite of this therapy, can occur in up to 20% of patients. Novel alternative therapies able to abrogate the aPL pathogenic action either by interfering with aPL binding at the placental level or by inhibiting the aPL-mediated detrimental effect are under active investigation.

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