What have we Learned Regarding Pregnancy Morbidity and Antiphospholipid Syndrome?

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Abstract: Women with antiphospholipid syndrome (APS) and antiphospholipid antibodies (aPL) are at a high risk of recurrent spontaneous miscarriages and late pregnancy complications. The prevalence of obstetric complications in aPL positive women is about 15-20%. The APS is defined by the combination of a clinical venous or arterial thrombotic event or a pregnancy complication and the laboratory detection of aPL. Adverse obstetric events include: recurrent miscarriages, late loss or early delivery due to preeclampsia or placental insufficiency. Although many of the clinical manifestations of the APS can be related to thrombotic events other non-thrombotic mechanisms have been proposed to explain the obstetrical manifestations. Though heparin and aspirin are indicated for the treatment of recurrent pregnancy loss, there is a paucity of randomized trials in this area. The therapeutic management of other obstetric complications, such as preeclampsia and intrauterine growth restriction need to be confirmed. New therapies for the prevention and the treatment of obstetric complication are being investigated in experimental models. This review will discuss of what we have learned regarding pregnancy morbidity and APS.

Keywords: Antiphospholipid antibodies, antiphospholipid syndrome, pregnancy loss, obstetric complications.

INTRODUCTION

There are few issues more interesting in our medical practice than the history of the antiphospholipid syndrome (APS). The APS was recognised as a separate entity in 1983. This full intriguing entity has gained the interest of every single medical area, such as rheumatology, haematology, dermatology, neurology, cardiology and many others. Recurrent spontaneous pregnancy losses tend to be the most common complication for which patients are referred to hematologists for suspected APS. Women with APS and antiphospholipid antibodies (aPL) are at a high risk for recurrent spontaneous miscarriages and late pregnancy complications. The prevalence of pregnancy complications in aPL positive women is about 15-20% [1]. While, not listed in the formal investigation criteria for APS, other complications that have been attributed to the condition, include intrauterine growth restriction (IUGR), oligohydramnios and the HELLP syndrome (haemolysis, elevated liver enzymes, and low platelet count).

Since the first description of the association of fetal death to the presence of a circulating anticoagulant [2], the study of aPL is mandatory in these patients. In spite of great advances in many areas of maternal and perinatal medicine during the last years, we still have few answers to explain many pregnancy complications such a recurrent abortions, fetal death, preeclampsia, or IUGR [3]. We can affirm that APS perfectly fits this empty space. The mechanisms involved in the obstetric compromise are actually debated and the question about the possible causal role of the aPL is still an unanswered question [4].

But what we must realise is that actually there is still a difficulty in the correct interpretation of the relationship between pregnancy complications and the APS and much more in defining therapeutic options. This observation raises from our daily medical practice, where in spite of the recently described obstetric criteria to define APS [5], aPL are measured in many patients with obstetric complications that no fulfil APS obstetric criteria. We must add to this observation that laboratory results are sometimes variable from one laboratory to another, positive criteria for laboratory test, such as anticardiolipin antibodies (aCL) differs from one centre to another and the lupus anticoagulant (LA) assay is not well standardised [6]. The presence of a positive aPL alone does not necessarily leads to a clinical event and women with high titers of aPL can have a normal obstetric outcome.

The aims of this chapter are to focus on what we have learned regarding pregnancy morbidity and APS. We will first define the syndrome, describe the changes in haemostasis and placental-interface and give a practical opinion from our daily practice, then we will refer to the pathogenic role of aPL in pregnancy complications and the current suggested treatment and finally, to the future directions of the obstetric APS.

DEFINITION OF THE APS

The APS is defined by the combination of a clinical criterion: venous or arterial thrombotic event (type1) or an obstetric complication (type 2) and the laboratory criterion (detection of aPL) (Table **1**). The syndrome is primary, when there is no evidence of an underlining disease and secondary mainly in the setting of systemic lupus erythematosus [5].

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Table 1. Revised Classification Criteria for the Antiphospholipid Syndrome

Antiphospholipid syndrome (APS) is present if at least one of the clinical criteria and one of the laboratory criteria that follow are met.

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 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 10^{th} 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 34^{th} week of gestation because of;
      i. eclampsia or severe preeclampsia defined according to standard definitions, or
      ii. recognised features of placenta insufficiency ((i) abnormal or non-reassuring fetal surveillance test(s), e.g. a non-reactive non-stress test, suggestive of fetal hypoxemia, (ii) abnormal Doppler flow velocimetry waveform analysis suggestive of fetal hypoxemia, e.g. absent end-diastolic flow in the umbilical artery, (iii) oligohydramnios, e.g. an amniotic fluid index of 5 cm or less, or (iv) a postnatal birth weight less than the 10^{th} percentile for the gestational age), or
   c. Three or more unexplained consecutive spontaneous abortions before the 10^{th} week of gestation, with maternal anatomic or hormonal abnormalities and paternal and maternal chromosomal causes excluded.

In studies of populations of patients, who have more than one type of pregnancy morbidity, investigators are strongly encouraged to stratify groups of subjects according to a, b, or c above.

Laboratory Criteria

1. **Lupus Anticoagulant** present in plasma, on two or more occasions at least 12 weeks apart, detected according to the guidelines of the International Society of Thrombosis and Haemostasis.
2. **Anticardiolipin Antibody** of IgG and/or IgM isotype in serum or plasma, present in medium or high titer (i.e. >40 IgG phospholipid units or IgM phospholipid units, or above the 99^{th} percentile), on two or more occasions at least 12 weeks apart, measured by a standardized enzyme-linked immunosorbent assay (ELISA).
3. **Anti-β_2 Glycoprotein I Antibody** of IgG and/or IgM isotype in serum or plasma, (titer above the 99^{th} percentile) present on two or more occasions at least 12 weeks apart, measured by a standardised ELISA.

Antiphospholipid antibodies are a heterogeneous group of immunoglobulins IgG, IgM or less frequently IgA. The association between aPL and pregnancy loss was first suggested more than 30 years ago [2]. Other pregnancy complications linked to placental insufficiency, such as preeclampsia and fetal growth restriction were included in the definition of the APS [7], so the adverse obstetric outcome included in the clinical criterion for the APS and their sub classification are: fetal death (type 2a), preeclampsia/placental insufficiency (type 2b) and recurrent early abortion (type 2c) [5].

Antiphospholipid antibodies do not recognize anionic phospholipids, as long believed [8]. They recognize plasma proteins bound to suitable anionic surfaces; among them, β_2-glycoprotein I, and prothrombin are the most common and investigated antigenic targets. β_2-glycoprotein I is required by the great majority of aCL to react with cardiolipin in immunoassays, whereas LA activity in phospholipid-dependent coagulation tests is caused by subgroups of both antibodies [9]. Other antigenic targets of the aPL can be activated protein C, protein S, human prothrombin, annexin A5, and endothelial protein C receptor [4]. Most reactions that control blood coagulation process occur at the phospholipid surface. The interaction of aPL with their respective antigens at the phospholipid surface may lead to a pro-thrombotic condition.

The aPL used to define the APS in Sapporo Preliminary Criteria were LA and aCL [7]. Amendments to the Sapporo Criteria were proposed at a Workshop in Sydney before the Eleventh International Congress on antiphospholipid antibodies in 2004. Anti-β_2-glycoprotein I ($a\beta_2$GPI) IgG and IgM assays are added in the revised criteria [5] (Table **1**). LA assays should be done according to the International Society of Thrombosis and Haemostasis (ISTH) guidelines. Recently, Pengo *et al.* have published an update of the guidelines for Lupus Anticoagulant detection as an Official Communication of the Scientific and Standardisation Committee on Lupus Anticoagulant/Phospholipid-dependent antibodies [10]. In our experience in pregnancy we found that the most sensitive screening test to detect LA was the Diluted Russell Viper Venom time [11].

HEMOSTATIC CHANGES DURING PREGNANCY, MATERNAL-FETAL INTERFACE, ROLE OF THE PLACENTA

Pregnancy is a hypercoagulable state [12]. Normal pregnancy is associated with an increase in clotting factors, a decrease of natural anticoagulants and an impairment of the fibrinolytic activity. There is an increase in the concentration of fibrinogen, factor VIII, V, VII, IX, X, XII and von Willebrand factor. Protein C ranges remains within the normal non-pregnant range, while Protein S decreases during pregnancy. An acquired activated protein C (APC) resistance has also been described during pregnancy and a reduction in the APC ratio has been associated to gestational vascular complications. There is an increase in the level of plasminogen activator inhibitor-2 (PAI-2) from placenta that leads to a

reduction of the fibrinolytic activity during the whole pregnancy but returns to normal after delivery [13]. The D-dimer is elevated during pregnancy and may reflect an increased coagulation activation and thrombin generation with an increased secondary fibrinolysis [14]. Finally an increased level of platelet and endothelial microparticles is observed during pregnancy. These microparticles have been associated to procoagulant complications. In conclusion, global normal haemostatic changes during pregnancy lead to an increase of the risk of thrombosis throughout pregnancy and may be linked to obstetric complications also.

On the other side, local haemostatic changes in placental vasculature have been well defined. An increased expression of tissue factor (TF) in the syncytiothrophoblast and a decreased concentration of tissue factor inhibitor (TFPI) pathway have been described [13]. Many hypotheses have been established in order to explain placenta mediated complications [15]. An interaction between the haemostatic, angiogenic and pro-inflammatory pathways is actually a focus of research and TF plays a central role in the connection between these different pathways. The production of TF by the decidual endothelial cells can promote uteroplacental thrombosis. During placentation trophoblast remodel decidual spiral arteries in high conductance vessels. During pregnancy, complications, such as preeclampsia there is a shallow trophoblast invasion that impedes decidual vascular conversion [16]. This abnormality leads to an inadequate uteroplacental flow. In preeclampsia there is an endothelial activation that induces hypoxia and reperfusion injury in the placenta. All these mechanisms together with apoptosis contribute to the pathology of preeclampsia and intrauterine growth restriction [17]. The coagulation system shared with the innate immunity an important role in the pathophysiology of obstetric complications. Thrombotic mechanism is only one more mechanism in this complex scenario. The possible pathogenic role of aPL in every single pathway that we have previously described confirms the importance of the aPL in the pathophysiology and probably causal relation of the APS with obstetric complications.

WHAT HAVE WE LEARNED ABOUT PREGNANCY MORBIDITY AND APS?

Early Pregnancy Complications/Recurrent Early Abortion

Early pregnancy loss is the most common complication of pregnancy and occurs in approximately half of all women. Most of these are clinically never recognised or there are only positive maternal biochemical serum markers. However, a significant number of pregnancies are lost during the first few weeks of early gestation [18].

Immune and coagulation systems play an important role together in the mechanisms involved in the early stages of pregnancy. Gestational complications present during early pregnancies can be divided in two parts: implantation failures and early abortion. These periods are the preembryonic periods that last from the conception to the fifth week and from this week begins the embryonic period. Some authors have reported that APS can cause preclinical pregnancy loss presenting as infertility but others have failed to find this association [19, 20].

Pregnancy is for the mother an external challenge and there is an important immune mediated inflammation during this period. The maternal-fetal interface must exhibit immune tolerance to the allogenic fetus. Several changes in the maternal immune response occur to protect the fetus from the maternal immune system. The aPL can interfere with this initial phase, inducing an increased apoptosis and trophoblast cell death [21, 22].

Once a successful embryonic implantation continues the possibility of an early pregnancy loss is faced with the genetic kariotype of the embryo. A chromosome abnormality is a frequent cause of early miscarriages, approaching near half of clinical losses [19]. In our experience the prevalence of APS in early recurrent loss patients (3 or more) is of 14-15% no more than the normal population[1]. The specificity of recurrent early abortion (Type 2c) criterion is uncertain because of the difficulty in excluding other known or suspected causes. The division between early implantation failure and early recurrent abortion has demonstrated to be important to define future therapeutic options.

Late Pregnancy Complications

Fetal Death

One of the earliest components in the setting of proper implantation and embryonic growth is the development of placental vasculature. Genetic abnormalities are much more infrequent (less than 2%) and aPL plays an important historical relationship with fetal death. One fetal death of a normal developed fetus is an obstetric criterion to define APS. Evidence from clinical experience and publications suggests that the fetal death (Type 2a) pregnancy morbidity criterion may be the most specific [5]. Fetal death is significantly associated to aPL, when this laboratory criteria is present. There is no doubt, when this event is present to indicate to the patient antithrombotic prophylaxis with heparin and aspirin in a new pregnancy. The specificity of the fetal death to define the APS is greater than that of early recurrent pregnancy loss [5].

Preeclampsia and Placental Insufficiency

Many studies have demonstrated a relationship between increased placental vascular resistance and abnormal fetal growth. The first trimester of pregnancy represents the most important period of diagnosis and treatment for many of the late-appearing pathologies of pregnancy. Several pathologies, such as the APS can affect the early development of a proper placental vasculature [23, 24]. The association between preeclampsia and APS is very interesting. We found a prevalence of LA in preeclampsia of 19% and in IUGR of 22%[2]. These two entities share many common pathogenic features. But we must realise that the preeclampsia/placental insufficiency (Type 2b) criterion may be relatively insensitive or non-specific. In its definition this criterion includes only cases requiring delivery before 34 week of gestation because of preeclampsia or placental insufficiency. Other cases with delivery before this week but for other reasons should not be included. The term placental insufficiency is

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not well defined and there are no specific histopathologic placental characteristics, either of APS or severe placental insufficiency [3]. The association of aPL with preeclampsia has been described by many authors [25] and this associations suggests that these antibodies should be investigated in such cases. So, there is few accurate information about pregnancy outcomes in women with preeclampsia and placental insufficiency associated to APS, there is an urgent need to enhance specificity by doing prospective clinical studies with a strict adherence to obstetric and laboratory criteria.

From 2003-2007, we prospectively evaluated women with obstetric criteria of APS (Sapporo criteria), laboratory tests confirmed APS in 67% of women with venous thrombosis, in 14% of early recurrent miscarriages, in 34% of fetal death and in 47% of women with preeclampsia or IUGR[1]. Only women with primary APS were included in this study.

DEFINING THE OBSTETRIC ANTIPHOSPHOLIPID SYNDROME: ARE WE IN THE CORRECT WAY?

Laboratory Criteria

In spite of the recent update of the guidelines for lupus anticoagulant and the efforts to define the laboratory criteria of the APS. Most of the information we have about obstetric prevalence and outcome of women with APS comes from studies that do not fulfil these criteria completely. In particular, aCL titres varies from one study to another and we do not have clear information about the confirmation of these values in a period of 12 weeks. There is still a debate about this issue [26] and there is not clear definition of the role of aCL antibodies in obstetric APS [27]. There are many studies, where aβ_2GPI were not tested because this test has been included as an APS criterion since 2006. The available information about the association between aβ_2GPI with pregnancy morbidity is controversial [28, 29]. Some studies have shown an association between aβ_2GPI of IgM isotype and recurrent abortion as well as *in vitro* fertilisation implantation failures [30]. Other studies showed a relationship between aβ_2GPI of IgG isotype and LA with two or more unexplained consecutive miscarriages, but only LA was associated with late pregnancy loss [30]. In contrast, other studies failed to show an association between aβ_2GPI with pregnancy complications. Today, there are many contradictory results on the clinical significance of aβ_2GPI in pregnancy complications. In our experience, testing for aβ_2GPI seems not to identify additional women with pregnancy complications in a group of patients, who have negative tests for LA and aCL[1]. The aβ_2GPI were positive only in pregnant women with venous thrombosis. Recently, Galli *et al.* [27] measured aPL to assess their clinical significance and found that LA positive patients who carried IgG aβ_2GPI and anti-annexin A5 antibodies (aAnA5) were at risk for both anamnestic abortion and prospective thrombosis. They suggest that this data supports the inclusion of aβ_2GPI in the laboratory criteria of APS and the removal de aCL. Alijotas-Reig *et al.* [31] in a cohort study that included 59 cases, found that only 16 patients with obstetric APS have repeatedly positive aβ_2GPI and suggest that these antibodies may be considered as a biological marker for obstetric APS. Although, some of the recent papers suggested that the detection of aβ_2GPI is associated with an increased risk of pregnancy loss not all authors agree that these obstetric-related manifestations are associated with them. Other antibodies against different phospholipids, such as anti-phosphatidyletanolamine [32] and aAnA5 [27] need to be validated in the clinical practice. For clinical purposes, it is important to find which aPL markers have the strongest association with pregnancy morbidity and best prognostic value. Large scale studies with standardised methodology are needed.

Clinical/Obstetric Criteria

Obstetric criteria must be properly defined. Some reports from the literature summarise women with only two early pregnancy losses. As previously mentioned confounding factors, such as chromosomal aberrations must be excluded, and the results of the treatment must be corrected, if a chromosomal aberration is detected [33]. Documentation of early recurrent loss, when it is possible, is necessary in order to obtain more information. Other causes of early recurrent loss must be studied. Placental morphology, cord anomalies and histopathology studies of the placenta and the fetus can be helpful in detecting other causes of fetal death [34].

PREGNANCY COMPLICATIONS IN APS: PATHOGENIC MECHANISMS

In the last few years, an important number of publications has provided new information regarding the novel pathogenic mechanism of antiphospholipid antibodies. Several thrombotic and non-thrombotic mechanisms have been proposed to explain aPL mediated obstetric complications [4]. Whether, there is a different mechanism when the obstetric complication comprises the early period of embryonic development and placentation or the late period, is unknown [20].

After implantation two developmental processes are crucial for placental development: trophoblast invasion and vascular remodelling. Decidual natural killer cells (NK) are required to support placental development, the depletion of uterine NK cells *in vivo* in mice induced important changes in post-implantation processes [35]. The placenta is a highly vascularised organ with a blood supply from fetal and maternal circulations. Maternal blood flows in the intervillous spaces, while fetal blood is confined in the intra-villous blood vessels. During normal placentation, the trophoblast cells invade deep into the maternal decidual tissue differentiating into extravillous trophoblast cells. These cells invade and remodel the maternal spiral arteries and differentiate into endovascular trophoblast cells. There is an increase of blood flow into the placental intervillous space. During pregnancy complications, such as spontaneous abortions and preeclampsia, this invasion is shallow with an insufficient transformation of the maternal vasculature. At the maternal-fetal interface, there is an influx of inflammatory immune cells with an alteration of the phenotypes of the local immune cells. The trophoblast synthesises its own β_2GPI and this protein translocates to the cell surface and can also bind exogenous β_2GPI on its cell surface [36]. This made the placenta a mayor target for aPL [37].

Pregnancy failures in patients with APS were initially associated with intraplacental thrombosis. Studies of placentas from women with APS showed thrombosis and infarction; however such histological finding is not specific for APS and was present in other conditions [38, 39]. So as pre-

viously described, aPL mediated thrombosis is not the only pathogenic mechanism of APS. The heterogeneity of the histological lesions supported other pathogenic mechanism [40, 41].

The idea that the thrombotic event at the maternal-fetal interface was the main pathogenic mechanism was supported during many years and confirmed by the successful outcome in patients treated with heparin. There is evidence from *in vitro* studies that aPL may induce a procoagulant state at the placental level. In physiologic conditions a crystal shield of annexin A5 is suggested to cover thrombogenic anionic surfaces preventing the activation of the coagulation cascade. This anticoagulant annexin A5 crystal shield can be disrupted by aPL that binds β_2GPI [42]. This has been showed in *in vitro* studies and the effect was also reproduced on trophoblasts [43]. Rand *et al.* [44] have proposed that the mechanism of recurrent pregnancy loss may be a consequence of aPL-mediated disruption of annexin A5 binding to syncytiotrophoblasts. This finding would impact on the modulation of coagulation within the placenta, and predispose to intervillous clot formation and subsequent diminished capacity for nutrient and oxygen exchange. In a recent study, aAnA5 were significantly increased in women with recurrent spontaneous miscarriage and recurrent implantation failures after *in vitro* fertilisation [45]. It has also been suggested that the endothelial protein C receptor (EPCR) plays a relevant role in pregnancy maintenance, since EPCR knock out mice experience placental thrombosis and early embryonic mortality [46]. The EPCR is a potentially important target of aPL, it has been hypothesised that antibodies directed against EPCR may be involved in the development of the thrombotic and obstetric complications of the APS [47].

Normal placentation can be altered by aPL, these antibodies might display a direct effect on maternal decidua and invading trophoblast. These effects are not related to thrombotic mechanisms and can explain pregnancies failures in early pregnancy development. The aPL can induce a defective endovascular trophoblast invasion [40], apoptosis, decreased human gonadotrophin production and inhibition of syncytia formation and proliferation [48]. *In vitro* models of trophoblast invasion show that aPL can regulate the expression of adhesion molecules.

Recent studies have suggested that activation of the complement cascade is necessary for aPL-mediated fetal loss. Mice deficient in complement component C3 and C5 were resistant to thrombosis, fetal loss and endothelial activation induced by aPL [49]. In patients with recurrent pregnancy loss associated with APS an impaired expression of endometrial differentiation markers and complement regulatory proteins suggested that these aPL antibodies can affect normal implantation and predispose to complement-mediated pregnancy failure [50]. Acute inflammatory changes during this phase are responsible for pregnancy failure. The role of TNF-α in pregnancy complications associated with aPL was examined in a murine model of APS [51]. Soluble proinflammatory mediators, such as cytokines complement and chemokines play a role in animal models. Several studies have shown that either immunisation with β_2GPI [52], or the passive transfer of aPL in mice promotes fetal resorption, fetal death and IUGR with placental inflammatory damage [53, 54]. As complement C3 is required for aPL-induced fetal loss, protection from fetal death and resorption was observed by the inhibition of the complement cascade or in C3-deficient mice [55]. It has also been shown that an interaction between the complement component C5a and its receptor (C5aR) is necessary [56]. Additional studies showed that null β_2GPI -/- mice were resistant to this antibody induced effect and confirm the importance of β_2GPI in the pathogenesis of APS. The presence of β_2GPI on trophoblast cell surfaces is a prerequisite for supporting the direct pathogenic effect of aPL [54].

All these findings suggested that inflammation may be responsible of aPL-induced pregnancy loss rather than thrombosis at the maternal-fetal interface. Placentas from patients with APS exhibit increased C4d and C3b deposition in the trophoblast, suggesting excessive complement activation associated with placental injury [57]. The deposition of complement on placentas has not been confirmed in other prospective studies [58]. The advance in the knowledge of pathogenic mechanisms triggered by aPL can be useful for the investigation of the targeted therapies in obstetric complications in APS [59].

TREATMENT OF THE OBSTETRIC APS

The APS is an established and treatable cause for recurrent miscarriage. The mechanism of aPL associated pregnancy loss is related to the deleterious effect of these antibodies on embryonic implantation, trophoblast function and differentiation [20-22] and placental thrombosis [60]. Venous or arterial thrombosis can be developed during the whole pregnancy and the postpartum period. Women with pregnancy loss and APS can have a poor live birth rate in the future untreated pregnancies [61].

Various interventions have been recommended to prevent recurrent miscarriages and maintenance of the pregnancy until delivery of a live infant of a mother with APS. The first successful treatment, reported by Nilsson in 1975, involved a preterm caesarean section in a woman with three previous fetal losses [2]. The combination of prednisone and aspirin was reported to be successful in a case series, but concerns with respect to the effect of prednisone on mother and the child limited this treatment. The successful use of intravenous immunoglobulin (IVIG) therapy was published by Carreras *et al.* [62] in 1988 and the use of plasmapheresis in APS was first reported by Kobayashi [63], but these treatments are not free of adverse effects, such as thrombosis with the use of IVIG or infections in plasmapheresis. Other alternative therapy is low dose aspirin.

Rosove *et al.* reported the first use of unfractionated heparin therapy (UFH) (1990) [64]. Actually UFH or low molecular weight heparin (LMWH) alone or together with low doses of aspirin is being used in many patients to prevent pregnancy miscarriages associated to aPL and APS. Small randomised studies showed the benefit of heparin and aspirin over aspirin alone in patients with recurrent miscarriages and this is the current recommended management. The treatment with combination of aspirin and heparin significantly improves the livebirth rate in these women [65, 66]. During the initial weeks of gestation heparin can improve trophoblast invasion and differentiation resulting in successful implantation [21]. In late pregnancy the anticoagulant effect can reduce placental thrombosis. The objectives of

heparin treatment are the prevention of maternal thrombosis and the improvement of gestational outcome. But in spite of the continued efforts in understanding the pathogenesis of obstetric complications of APS, there are few therapeutic trials.

The treatment of recurrent miscarriages attributed to the APS has been recently reviewed in an analysis of 13 studies that included 849 patients [67-69]. The authors observed that the available studies had significant limits: small trial size, absence of blinding, lack of no treatment arm and highly variable entry criteria, treatments and endpoint definitions leading to trial clinical heterogeneity. The authors concluded that combined UFH and aspirin may reduce pregnancy loss by 54% and that large randomised controlled trials with adequate allocation concealment are needed to explore potential differences between UFH and LMWH [69]. Most clinicians currently treat the patients with a combination of prophylactic dosage of LMWH plus low dose aspirin. The American College of Chest Physicians Evidence-Based Clinical Practice Guidelines recommend for women with aPL and recurrent (three or more) pregnancy loss or a late pregnancy loss and no history of venous or arterial thrombosis, antepartum administration of prophylactic or intermediate-dose UFH or prophylactic LMWH combined with aspirin [70]. Aspirin is usually indicated during the preconceptional period and heparin when pregnancy is documented. Platelet counts should be monitored; although heparin induced thrombocytopenia is very rare in pregnancy. As we previously mentioned, this recommendation lies in the results of few trials with important differences in trial design. Whilst this treatment is reasonable, aspirin alone or a watch and waiting approach can be defended particularly in patients with previous pregnancy early recurrent losses [71]. Some studies showed that the chance of a successful pregnancy even without treatment is still favorable [72]. We treated patients with prophylactic doses of enoxaparin 40 mg daily and 100 mg of aspirin and live birth occurred in 39/49 (79%) treated pregnancies[3].

During the last month of pregnancy treatment is usually modified, in some cases heparin is stopped, in others some clinicians switch to UFH because of its short life and in others LMWH is continued and a programmed delivery is planned. Heparin is generally resumed postpartum in order to reduce the risk of postpartum vein thrombosis. The recommendations about the period to continue prophylactic heparin during the postpartum in women with obstetric APS without thrombosis differs from one author to another. Clark *el al* observed a very low rate of postpartum thrombosis in patients with recurrent miscarriages and aPL and no history of thrombosis [73]. We recommend continuing heparin for a period of 4-10 days in patients with aPL without previous thrombosis and without other risk factors[4]. In the presence of other risk factors such as other autoimmune diseases, immobilisation or obesity heparin can be continued for a longer period. In cases of previous venous thrombosis heparin must be resumed for a period of 6 weeks. We agree with other authors that there is still insufficient evidence on which to base recommendations for thromboprophylaxis during pregnancy and the early postnatal period for women with aPL and no history of thrombosis [71]. An unresolved issue is the duration of the heparin treatment. In many pregnancies, the continuation of heparin until delivery is reasonable, but as many miscarriages occurred before 13 weeks and the live birth of pregnancy that reaches 14 weeks of gestation is high, some authors suggested that heparin treatment could be suspended after this time [74]. Uterine artery flow waveform analysis can be a useful aid to screen women at high risk of uteroplacental insufficiency. In cases of recurrent first trimester miscarriages and normal uterine artery doppler studies, some authors suggested that cessation of LMWH may be considered at 20 weeks gestation [75, 76].

Other results described by Empson *et al.* were that there was no advantage in high dose over low dose UFH; that aspirin alone showed no significant reduction in pregnancy loss in three trials as compared with placebo; that prednisone and aspirin resulted in a significant increase in prematurity and diabetes, when compared to placebo, aspirin, and aspirin combined with heparin, but no significant benefit, and that IVIG was associated with an increased risk of pregnancy loss or premature birth, when compared to UFH or LMWH combined with aspirin; when compared to prednisone and aspirin IVIG was not significant different in outcomes [69]. In spite of these results a small benefit to aspirin alone or of IVIG cannot be excluded on the basis of the available studies [77]. The studies with IVIG are small and further studies are required. The treatment with heparin is generally extrapolated to other APS associated pregnancy complications.

The significant improve of outcomes of pregnancies with APS treated with heparin can also be explained by other mechanism, in addition to its anticoagulant effects. Di Simone *et al.* demonstrated that LMWH was able to reduce aPL binding to trophoblast cells and to restore *in vitro* placental invasiveness and differentiation [21]. Other roles of heparin are under investigation. Heparin might protect pregnancies by reducing the binding of aPL, reducing inflammation, modulation of trophoblast apoptosis, promotion of trophoblast cells invasiveness, facilitating implantation and/or inhibiting complement activation [4, 78]. Neither fondaparinux nor hirudin, or other anticoagulants without known effects on complement prevented pregnancy loss, demonstrating that anticoagulant therapy is insufficient protection against APS. It is still unknown if heparin can directly affect placental functions o plays its role by an enhancement of the activity of specific proteases, such as metalloproteinases involved in trophoblast invasion into the endometrial tissues [21].

The standard anticoagulant therapy has dramatically changed the prognosis of the APS. In spite of this treatment obstetrical recurrences are seen and a high rate of complications related to placental vascular complications, such as preeclampsia and fetal growth retardation may be observed in the treated pregnant women [79][5]. This arise the question whether additional therapeutic strategies should be used. As it has been recently described, the proinflammatory response of the trophoblast to aβ_2GPI is mediated by the Toll-like re-

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ceptor 4 pathway [59, 80]. This mechanism might be used to treat aPL related pregnancy complications. As previously described, aPL that activate complement induce TF and fetal death, so an attractive option is to inhibit complement activation to treat or ameliorate clinical manifestations of APS.

CONCLUSIONS AND COMMENTS

In spite of the international efforts to standardise laboratory testing for aPL, there is a considerable inter and intra laboratory variation in their detection. Laboratory problems contribute to the controversies in our understanding and definition of this syndrome. We hope that in the near future, a better obstetric and laboratory definition of the APS, with different levels of risk, in well designed therapeutic trials, will bring us better evidence regarding the management of obstetric APS. At the present time testing for aPL should usually be restricted to patients, who have thrombosis or pregnancy complications that may be attributable to APS. Patients must be properly defined. Testing should be done according to updated recommendations and persistence of the abnormal tests should be confirmed after 12 weeks. However, there remain many unanswered questions regarding treatment with heparin. Several mechanisms could explain the beneficial effects of heparin in addition to its anticoagulant effect. Heparins exert a number of important actions that could have beneficial effect but there is severe lack of adequate clinical trials to adequately define heparin's role in preventing pregnancy outcomes. Clinical trials must need to be done to validate these findings in humans. Finally, we need to translate recent research and experimental observations to the clinical practice in order to change the poor obstetric outcome in women with obstetric complications associated to APS. Understanding the molecular pathogenesis of aPL mediated thrombosis may help to discover new therapeutic tools to prevent and treat pregnancy morbidity associated with APS.

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