Anti-phospholipid antibodies and pregnancy

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A B S T R A C T

The presence of antiphospholipid antibodies is linked to an increased rate of repeated spontaneous abortions and fetal deaths, that in fact belong to the clinical spectrum of the antiphospholipid syndrome. The pathogenic role of antiphospholipid antibodies was clearly shown in experimental animals that, when infused during pregnancy, develop placental insufficiency and miscarriages. In addition, *in vitro* aPL were shown to bind trophoblastic cells and to impair their function. However, since pregnant patients with this condition were appropriately managed, APS was defined as one of the few tractable cause of pregnancy losses. In fact, despite a significant number of complications still recorded, the large majority of these pregnancies now end with life births. Data on perinatal and long-term outcome of children born to patients with antiphospholipid antibodies are reassuring.

Key words: Anti-phospholipid antibodies, anti-cardiolipin antibodies, lupus anticoagulant, anti-β2 glycoprotein I antibodies, pregnancy.

The antiphospholipid syndrome; definition

After the formulation of the international preliminary classification (Sapporo) criteria for antiphospholipid syndrome (APS)[1] (Table 1), a pre-conference workshop, preceding the Eleventh International Congress on Antiphospholipid Antibodies (aPL),in Sidney, November 2004, revised the international classification criteria for APS. Probably the most relevant change is that now IgG and IgM anti-B_2 glycoprotein I (anti-B2GPI) assays are added in the revised criteria (Spiros M *et al.,* submitted).

Clinical and laboratory features not included in the revised classification criteria for APS (livedo reticularis, thrombocytopenia, heart valve disease) are undoubtedly frequent in patients with APS, but concerns exist regarding specificity. Rejection of those features as independent classification criteria does not mean that their association with APS is unrecognized. Finally, the committee advised against using the term *secondary* APS: rather than distinguishing between patients with *primary* and *secondary* APS, documenting the coexistence of systemic lupus erythematosus (SLE) or other disease is more appropriate for classification (Spiros M *et al.,* submitted). Definite APS requires the combination of at least one clinical and one laboratory criterion.

It must be underlined that the criteria are intended to ensure uniform characterization of patients for clinical studies. These criteria are not intended for diagnosis or treatment of individual patients. In routine clinical practice, clinicians may diagnose as APS patients who do not fulfill the criteria. Prospective studies examining disease and/or treatment outcome, however, should include patient groups defined and classified according to the present criteria.

Clinical experience and limited published data suggest that the fetal death Sapporo pregnancy morbidity criterion is the most specific for APS; recurrent early abortion is less certain because of the difficulty in excluding other known or suspected causes. For women with recurrent early abortions, chromosomal abnormalities and other accepted causes of pregnancy loss should be examined. The Sapporo criterion for preterm birth due to severe preeclampsia or placental insufficiency included cases less than 34 weeks' gestation to enhance specificity. Substandard performance of this criterion results from inclusion of any preterm birth due to preeclampsia or placental insufficiency. The committee emphasizes adherence to strict definitions of severe preeclampsia[2] and placental insufficiency.[3] The Sidney workshop found no advantage to removing the preeclampsia-

Table 1. Sapporo Criteria for the antiphospholipid syndrome.

CLINICAL CRITERIA

VASCULAR THROMBOSIS

one or more clinical episodes of documented arterial, venous or small vessel thrombosis

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: (a) eclampsia or severe preeclampsia defined according to standard definitions, or (b) recognised features of placental insuffi ciency, 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

ANTICARDIOLIPIN ANTIBODY

(IgG and/or IgM, at medium or high titre, on 2 or more occasions, six weeks or more apart, measured by a standardized ELISA for β2GPI-dependent aCL).

LUPUS ANTICOAGULANT

(according to the guidelines of the International Society on Thrombosis and Hemostasis Scientific Subcommitee on Lupus Anticoagulants/phospholipid dependent antibodies).

placental insufficiency criterion but strongly encouraged investigators to include only cases requiring delivery before 34 weeks.[2] The Committee recognized that there is no widely accepted or standard definition for placental insufficiency and that timing of delivery is subject to physician judgment, but common definitions include: abnormal or non-reassuring fetal surveillance test(s); abnormal Doppler flow velocimetry waveform analysis, absent end-diastolic flow in the umbilical artery; oligohydramnios; or a post natal birth weight less than the 10th percentile for the gestational age.[2,3] Furthermore, there are no specific histopathologic placental abnormalities characteristic of either APS or *severe* placental insufficiency.[4] The Sidney Committee suggested that the criterion for placental insufficiency be any of the above clinical criteria associated with the decision of a qualified clinician to deliver a morphologically normal fetus prior to 34 weeks (Spiros *et al.*, submitted).

Pathogenesis of fetal loss

Antiphospholipid antibodies (aPL) are recognized as the most frequent acquired risk factor for recurrent thrombosis and as a treatable cause of recurrent pregnancy loss.[5] It is now clear that aPL are a heterogeneous family of autoantibodies detectable by different assays such as Lupus Anticoagulant (LA), anti-cardiolipin (aCL) and anti-β2 glycoprotein I solid phase assays. A significant association between recurrent miscarriages and pregnancy complications has been reported with all the above mentioned assays.[6] Several pathogenic mechanisms have been suggested to explain the APS-associated obstetrical manifestations.[7]

Intra-placental thrombosis with maternal-fetal blood exchange impairment was traditionally suggested to be the main pathogenic event. A substantial body of evidence from the histological examination of placentas obtained from APS women and from *in vitro* studies supported the hypothesis that thrombosis might play a role. The original description of widespread thrombosis and infarction of the placenta obtained from a woman with APS was actually confirmed by others who investigated both first and second trimester placentas.[8-10] In line with such a hypothesis are all the studies that demonstrated the ability of aPL to induce a general pro-coagulant state.[11]

Annexin V is a 35 kD natural anticoagulant plasma protein able to bind anionic phospholipids exposed on the surfaces in close contact with the blood; it has been suggested that a shield of Annexin V might make the anionic surfaces non-thrombogenic by preventing the binding of activated factor X and prothrombin. Rand *et al.*, reported that women with aPL have significantly lower distribution of Annexin V covering the intervillous surfaces of their placentas in comparison with normal controls.[12] The reduction of the Annexin V shield was suggested to be responsible for the thrombophilic state of the syncytiotrophoblast surface. Although experimental models emphasized the role of placental thrombotic phenomena, epidemiological studies reported that thrombotic events cannot account for all the miscarriages and the histopathological findings in the placentas from APS women.[13] In addition, it has been suggested that intraplacental

thrombosis is unlikely to be responsible for first trimester pregnancy losses, and that abnormalities of early trophoblast invasion may be the primary pathological mechanism in such cases.[14]

Evidence from *in vitro* APS experimental models suggests that aPL might display a direct effect on trophoblast by impairing its differentiation/maturation through mechanisms unrelated to thrombosis, and that might be inhibited by heparin. It has been actually reported that aPL binding to trophoblasts might end into direct cellular injury, apoptosis, inhibition of proliferation and syncytia formation, decreased human chorionic gonadotrophin (hCG) production and defective invasiveness.[15, 16] The demonstration of the presence of β2GPI on the trophoblast cell membranes explains the aPL placental trophism, being β2GPI one of the most important antigenic target for aPL. It has been suggested that β2GPI, as a cationic plasma protein, might bind to phosphatidylserine exposed on the external cell membranes of trophoblasts undergoing to syncytium formation. *In vitro* studies with both murine and human monoclonal as well as with polyclonal IgG antibodies from APS patients clearly demonstrated a binding to trophoblast monolayers.[15, 17] These findings do explain why aPL passively infused in naïve pregnant mice rapidly disappear from the circulation and are entrapped in the placenta tissues.[18]

Interestingly, once bound, these antibodies can affect the trophoblast functions. Adler *et al.,* provided direct evidence that aPL were able to react with syncytiotrophoblast and to prevent intertrophoblast fusion,[19] while Rote *et al.,* showed that an anti-phosphatidylserine monoclonal antibody bound trophoblast cells and prevented their *in vitro* invasiveness and hCG secretion.[20] Di Simone *et al.,* has reported comparable results with spontaneously occurring polyclonal IgG fractions from APS patients as well as human IgM monoclonal antibodies with anti-β2GPI activity.[15]

In line with these findings are the data showing that an anti-β2GPI monoclonal antibody affected human choriocarcinoma cell line proliferation *in vitro*.[21]

Normal trophoblast invasion is a dynamic process which is tightly controlled via a complex series of interactions between trophoblast and decidual tissues. The differentiation of trophoblast into an invasive phenotype is related to the expression of cell surface adhesion and signalling molecules. Using an *in vitro* model of trophoblast invasion, Di Simone *et al.,* has recently demonstrated that aPL might affect placental invasion also through an abnormal trophoblast integrin and cadherin expression.[22] It is likely that the trophoblast failure to express the right adhesion molecule phenotype could tip the delicate balance that physiologically favours decidual invasion. As a whole, these findings do suggest that aPL may be pathogenic in affecting several trophoblast functions eventually ending into a defective placentation without necessarily involving thrombotic phenomena. β2GPI binding to trophoblast cells might have a pivotal role in this mechanism.[23] Recent work has suggested that complement activation may also contribute to fetal loss[24,25] which is rather surprising as the classical pathology found in the aPL syndrome is a bland non-inflammatory thrombosis[26] and complement activation typically results in an inflammatory pathology.

Treatment

A peculiar aspect of pregnancy is that it starts, it lasts about 40 weeks and it ends with the potential of two-hundred per cent of morbidity and mortality (mother and fetus). Therefore is one of the few situation where the physician, when consulted, can really understand his/her own success or failure. This is a relatively unusual situation for specialists devoted to the care of chronic diseases, like SLE and other autoimmune conditions.

Our understanding of the possible mechanism of action of aPL has led to two treatment modalities.[27] The first one is focused on reducing the production of antibodies mainly with steroids and intravenous immunoglobulin. The second alternative includes the use of antiaggregant/anticoagulant agents, mainly aspirin and heparin, to prevent thrombosis in the uteroplacental circulation. Low-dose aspirin may also improve placental blood flow by decreasing the thromboxane to prostacyclin ratio. These therapeutic agents have been used alone or in combination. Interventions with these drug therapies and monitored pregnancy have increased fetal survival, but no gold standard has been determined.

Available data are limited by the small number of patients in individual studies, which have also had varying entry criteria and treatment protocols, and by the lack of standardization of laboratory assays used to detect aPL . However, since pregnant patients with this condition were appropriately managed, APS was defined as one of the few tractable cause of pregnancy losse.[28] In fact, despite a significant number of complications still recorded, the large majority of these pregnancies now end with life births.

Aspirin

Aspirin was nearly always added, when corticosteroids were the mainstay of treatment. In addition to its effects on platelet aggregation and thromboxane-prostacyclin balance, low-dose aspirin has been found to significantly reduce the fetal resorption rate in the experimental APS.[29] Aspirin inhibits irreversibly the

synthesis of thromboxane A_2, a potent platelet aggregate. Data in the literature supporting the beneficial vasodilating effects of aspirin in conditions as diverse as intrauterine growth retardation with umbilical placental insufficiency to preeclampsia and thrombosis secondary to platelet aggregation, all support the idea that treatment with aspirin will help to prevent the vascular and thrombotic complications associated with aPL.[30] Good results with low-dose aspirin alone, with success rates over 70%, have been achieved by several groups in APS patients with two or more pregnancy losses.[31-38] Aspirin daily doses used in these studies ranged between 75 mg and 100 mg. The optimal antiaggregant dose for aspirin is still uncertain. Although doses as high as 325 mg three times a day have been used in the past, there is no evidence that doses higher than 75 mg/day are more effective in preventing thrombotic events, whilst toxicity is probably dose-related.[39]

Potential complications of aspirin during pregnancy include birth defects and bleeding in the neonate and in the mother. However, according to meta-analyses and large trials these potential effects on the mother and her infant appear at doses averaging 1,500 mg/day, but not at doses ≤150 mg/day.[40, 41] Thus low-dose aspirin (≤150 mg/day) during pregnancy is safe for the mother and fetus. However, aspirin treatment has to be discussed in patients with abnormal platelet function, low platelet counts, or with hemorrhagic diseases.[27]

Anticoagulants

The use of heparin was a logical approach to treatment for a disorder resulting from thrombosis. Furthermore heparin might reverse some negative effects of aPL on trophoblast gonadotrophin secretion and invasiveness.[15] Heparin has been reported to inhibit the binding of aPL to their target and to absorb aPL *in vitro*. Heparins are highly negatively charged molecules, and these *in vitro* effects are not surprising. It is not clear whether these effects are important *in vivo*, in patients with aPL. In the earliest published case series in 1990,[42] it was observed that under heparin (mean dose 24,700 U/day), 14/15 pregnancies ended in live births in 14 women with aPL and history of 28/29 miscarriages. Over the past decade, several case series recounted a live-birth rate of ~70-75% in women treated with unfractionated heparin, alone or in combination with low-dose aspirin (60-100 mg/day). Although some authors used sufficient doses to achieve full anticoagulation, equivalent results were achieved with prophylactic doses.[39,43, 44]

There is now accumulating experience with the use of low-molecular-weight heparins both in pregnant and nonpregnant patients for the prevention of complications associated with aPL and there is also evidence that low-molecular-weight heparins do not cross the placenta and they are safe and effective in pregnancy.[45, 46] A systematic review of the available evidence[46] has analyzed 486 pregnancies (163 with aPL and/or other autoantibodies) treated with low-molecular-weight heparins (nadroparin, enoxaparin, dalteparin, reviparin and tinzaparin) as the only anticoagulant. This review has demonstrated this group of drugs to be very effective, with only three cases of thromboembolic complications reported and no episodes of major bleeding. This is noteworthy considering that pregnancy is a high-risk period for thrombosis and all women with a previous history of thrombotic events must receive thromboprophylaxis with full anticoagulation during this period.

Oral anticoagulants cross the placenta, are teratogenic (chondrodysplasia punctata) and must, therefore, be avoided during the first trimester. The period of risk is between the sixth and twelfth week of gestation, so conception on coumadin derivatives is not dangerous provided that these drugs are replaced with heparin within two weeks of the first missed period.[47] Therefore, in patients of high risk of recurrent thrombosis during pregnancy (those with previous arterial disease, mainly stroke), oral anticoagulants can be reintroduced in the second trimester if necessary. Coumadin is also used for postpartum thromboprophylaxis. The above notwithstanding, oral anticoagulants cause an anticoagulant effect in the fetus that is a concern, particularly at the time of delivery, when the combination of the anticoagulant effect and trauma of delivery can lead to bleeding in the neonate.[45]

Low-molecular-weight heparins have potential advantages over unfractioned heparin during pregnancy because they cause less heparin-induced thrombocytopenia, have the potential for once-daily administration because of better bioavailability and longer half-life, and may result in a lower risk for heparin-induced osteoporosis.[45] Prolonged heparin therapy in pregnancy has been associated with osteoporosis and vertebral collapse, especially when used in combination with prednisone (mainly at high-dose) and thus some authors suggest that this combination should not be used.[43] Administration of extra oral calcium (1000 mg/day) may help to minimize heparin-induced osteopenia.[48]

Which protocol?

The mainstay of treatment now rests with antiplatelet and anti-thrombotic treatments but the question of choice between heparin alone or with aspirin versus aspirin alone remains controversial. The current

most recommended treatment for women with recurrent pregnancy wastage and aPL is heparin and low-dose aspirin starting therapy when pregnancy is confirmed.[43,48,49] This recommendation is essentially based on two clinical trials which have found better obstetric outcomes using aspirin plus heparin than aspirin alone.[50,51] The study by Rai *et al.,*[50] was a randomized trial, but Kutteh[5] assigned treatment in a consecutive way, which limits the validity of his results. Results from both studies, however, were quite similar. Kutteh[51] alternatively assigned aspirin (81 mg/day) or aspirin plus 10,000 U/day heparin in 50 women with aCL. The live birth rate in the heparin treated group was 80% versus 44% in women treated with aspirin alone. Rai *et al.,*[50] compared aspirin with heparin 10,000 U/day plus aspirin in 90 women. The live birth rate was 71% with heparin treatment versus 42% with aspirin alone. In both studies no differences were found between treatment groups with respect to obstetrical complications. No case of thrombocytopenia or thrombosis occurred but women receiving heparin plus aspirin had a median decrease in lumbar spine bone density of 5.4%.[50] Potential limitations of these two studies have been previously stressed.[52]

Therefore, on the above evidence, it seems clear that aspirin has a place in the treatment strategy of pregnancy losses associated with the APS and doubt as to whether heparin is always needed comes from the experience of several groups of investigators showing marked improvement in pregnancy rates during treatment with aspirin alone as compared with previous reproductive performance in the same women.[33-37,53] An important part of such improvement of prognosis in these patients is thought to be due to better obstetric surveillance. Indeed, a recently published double-bind, randomized, placebo-controlled trial,[54] including 40 women with aPL and recurrent miscarriage, has not shown any benefit of adding aspirin to an intensive obstetric care and placebo treatment. The prognosis in both the aspirin and control groups was remarkably good, with success rates over 80%. However, it is noteworthy to note that treatment was started when pregnancy was diagnosed or on discovery of aPL during pregnancy but not before conception. On the other hand, most patients recruited for the study had only low-titer aCL and most important, emotional support and continuity of personnel were provided, including a liberal admission policy. Similar success rates with supportive care have been previously reported in women with unexplained recurrent miscarriage.[55]

That study[55] thus emphasizes a very important aspect in the management of these patients and the only one where general agreement is found: close fetal and maternal surveillance by a well coordinated multidisciplinary team including obstetricians, internists/ rheumatologists, and hematologists.

Perinatal and long-term outcome of children born to patients with antiphospholipid antibodies

A case control study, recently performed by Tincani *et al.,*[56] focused on babies from mothers with primary APS compared with babies from healthy mothers. The 2 groups were matched for gestational age and pregnancy complications, to verify if the presence of aPL was linked to specific risks for the fetus or the neonate. Despite the number of newborns (71 cases and 71 controls) consecutively examined, no significant difference was found in the occurrence of neonatal complications. However, in children from mothers with APS, case reports of neonatal thrombosis were recorded, involving brain or other districts.[57, 58] Lojacono *et al.,*[59] also described a fetal stroke associated with maternal aPL, that was found, by ultrasound and CT scan, at 2 months of age, in the cerebral artery territory, likely due to an intrauterine event. Obviously these extremely rare events can have severe permanent consequences. There are very few data about the long-term outcome of children born to patients with antiphospholipid antibodies. Studying children born to SLE patients, Neri *et al.,*[60] recently reported that the occurrence of learning disabilities seems increased in subjects whose mothers were aPL positive. This observation is consistent with what reported in animal models, where a prolonged exposure to aPL can cause hyperactivity and anxiety[61] and with the *in vitro* data showing that aPL can bind brain tissue and brain endothelial cells.[62] On the other hand, an increased occurrence of learning disabilities was already reported in children born from SLE patients[63,64] and aPL might be considered at least part of the pathogenic factors responsible of them. Interestingly, the children with learning disabilities described by these groups have a normal intelligence level, therefore an early understanding of their problems could help to overcome possible difficulties during the school years.[65]

aPL antibodies in normal people and in SLE

Hard data deriving from prospective controlled studies are lacking. aPL antibodies occur in 1 to 5 percent of healthy young people; prevalence increases with age and presence of chronic disease.[66, 67] More than 30% of patients with SLE have aPL;[66, 68] aPL are frequent in patients with infectious diseases.[69, 70]

APS has been reported in 50 to 70 percent of patients with both SLE and aPL after 20 years of follow-up in some studies[71,72] while others have observed that the

progression to clinical SLE occurs only rarely in patients who were originally diagnosed as having primary aPL syndrome.[73] It has been showed in a retrospective observational study that APS women with only pregnancy morbidity where somehow protected by a long lasting therapy with low dose aspirin, while women who were not treated with low dose aspirin developed an high thrombosis rate over following years.[74]

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