Anti-phospholipid antibodies and pregnancy

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, \textit{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-\(\beta_2\) 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-\(\beta_2\) glycoprotein I (anti-\(\beta_2\)GPI) assays are added in the revised criteria (Spiros M \textit{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 \textit{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 \textit{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. The committee found no advantage to removing the preeclampsia-
placental insufficiency criterion but strongly encouraged investigators to include only cases requiring delivery before 34 weeks. 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.

Furthermore, there are no specific histopathologic placental abnormalities characteristic of either APS or severe placental insufficiency. 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. 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. Several pathogenic mechanisms have been suggested to explain the APS-associated obstetrical manifestations. 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. In line with such a hypothesis are all the studies that demonstrated the ability of aPL to induce a general pro-coagulant state. 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. 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. In addition, it has been suggested that intraplacental

| Table 1. Sapporo Criteria for the antiphospholipid syndrome. |
|------------------------|------------------------|
| **CLINICAL CRITERIA**   | **LABORATORY CRITERIA** |
| VASCULAR THROMBOSIS    | ANTICARDIOLIPIN ANTIBODY |
| one or more clinical episodes of documented arterial, venous or small vessel thrombosis | (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). |
| PREGNANCY MORBIDITY    | LUPUS ANTICOAGULANT     |
| (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 | (according to the guidelines of the International Society on Thrombosis and Hemostasis Scientific Subcommittee on Lupus Anticoagulants/phospholipid dependent antibodies). |
| (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 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    | |
| ANTICARDIOLIPIN ANTIBODY | |
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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. 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. 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. These findings do explain why aPL passively infused in naïve pregnant mice rapidly disappear from the circulation and are entrapped in the placenta tissues.

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, while Rote et al., showed that an anti-phosphatidylserine monoclonal antibody bound trophoblast cells and prevented their in vitro invasiveness and hCG secretion. 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.

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

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. 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 pathogen-
Good results with low-dose aspirin alone, it was observed that under heparin effects are not surprising. It is thus low-dose 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.

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. 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.

**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. 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, 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. There is now accumulating experience with the use of low-molecular-weight heparins both in pregnant and nonpregnant patients for the prevention of complica-

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**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. This recommendation is essentially based on two clinical trials which have found better obstetric outcomes using aspirin plus heparin than aspirin alone. The study by Rai et al. was a randomized trial, but Kutteh assigned treatment in a consecutive way, which limits the validity of its results. Results from both studies, however, were quite similar. Kutteh alternatively assigned aspirin (81 mg/day) or aspirin plus 10,000 U/day heparin in 50 women with aPL. The live birth rate in the heparin treated group was 80% versus 44% in women treated with aspirin alone. Rai et al. 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%. Potential limitations of these two studies have been previously stressed.

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. 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 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.

That study 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., 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. Lojacono et al. 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. 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 and with the in vitro data showing that aPL can bind brain tissue and brain endothelial cells. On the other hand, an increased occurrence of learning disabilities was already reported in children born from SLE patients 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.

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. More than 30% of patients with SLE have aPL and aPL are frequent in patients with infectious diseases. 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 while others have observed that the
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progression to clinical SLE occurs only rarely in patients who were originally diagnosed as having primary aPL syndrome. It has been shown 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.
B. Canesi et al.


