CHARLES UNIVERSITY IN PRAGUE

THIRD FACULTY OF MEDICINE

Institute for mother and child care in Podoli

Kira Oleinikov

Antiphospholipid syndrome and recurrent abortions

Diploma thesis

Prague, February 2009

Author of diploma thesis: Kira Oleinikov

Master's programme of study

Advisor of the thesis: : **MUDr. K. Andelova**

Department of the advisor of the thesis: **Institute for mother and child in Podoli**

Date and year of defence: February 2009

Written Declaration

I declare that I completed the submitted work individually and only used the mentioned sources and literature. Concurrently, I give my permission for this diploma/bachelor thesis to be used for study purposes.

In Prague on March 4th, 2009 Kira Oleinikov

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Introduction

Over thirty years ago was published a first case report of a young woman with a recurrent history of miscarriages investigated with coagulation studies during pregnancy and found to have a circulating anticoagulant. Her fourth pregnancy was managed successfully with caesarian section.

Here is citation from the first case study described by Nilsson in 1975:

"*During the third pregnancy a coagulation defect was diagnosed which was characterized by prolonged coagulation times. This defect disappeared after the end of the pregnancy but returned during the fourth pregnancy. This time a circulating anticoagulant was found. The anticoagulant titre rose during the pregnancy from 1/2 to 1/10. . . The fourth pregnancy was terminated by caesarean section in the 34th week. . . The placenta was severely infarcted. It is postulated that the development of antithromboplastin during pregnancy may be a contributory cause of intrauterine death.*" [1]

But it was not until 1983 when Graham R.V. Hughes linked recurrent strokes with abortions and lupus anticoagulant. It was then published as Hughes syndrome**.**[2]

The clinical complexity of what is now is described as the antiphospholipid syndrome (APS) has grown along with an increased understanding of its pathogenesis. Possibility of prevention of maternal and fetal morbidity and recurrent abortions results in continued interest and efforts to better define diagnosis and therapy for the condition.

Definitions

Before discussing this subject in details here are some relevant terminology definition.

Primary antiphospholipid syndrome (APS) refers to the association between antiphospholipid antibodies (aPL) and adverse pregnancy outcome or vascular thrombosis. Adverse pregnancy outcomes include:

(1) three or more consecutive miscarriages before ten weeks of gestation,

(2) one or more morphologically normal fetal deaths after the tenth week of gestation

(3) one or more preterm births before the 34th week of gestation due to severe pre-eclampsia, eclampsia or placental insufficiency.

Where APS exists in chronic inflammatory disorders, such as systemic lupus erythematosus, it is referred as secondary APS [3].

Epidemiology of APS and aPL –associated recurrent abortions.

The antibodies against phospholipid (aPL) of a cell membrane include antibodies against cardiolipin (anti-cardiolipin antibodies) and β_2 glycoprotein I (those have strongest association with APS).

Prevalence of aPL in general population ranges between 1-5%. But only minority develop clinical features of antiphospholipid syndrome . There are data on incidence that reaches 5 new cases per 100,000 persons per year and the prevalence around 40-50 cases per 100,000 persons. The prevalence is higher among patients with SLE (about 30%) , deep venous thrombosis (30%) .[4]

Antiphospholipid antibodies are present in 15% of women with recurrent miscarriage. By comparison, the prevalence of aPL in women with a low risk obstetric history is less than 2%. In women with recurrent miscarriage associated with aPL, the live birth rate in pregnancies with no pharmacological intervention may be as low as 10%.[5]

Concerning the secondary aPL syndrome - between 20% and 40% of patients with systemic lupus erythematosus (SLE) have anti phospholipid antibodies, but less than half of these patients have APS.

Approximately 50% of patients who have APS also have SLE. [6]

In present time , antiphospholipid antibodies (aPL) are regarded as the most frequently acquired risk factor for thrombophilia and as a treatable cause for pregnancy loss. [9]

Due to the high rate of losses in the embryonic period that occur in the general population (with chromosomal abnormalities of the conceptus being the most frequent cause) and the rarity of losses in the fetal period in the general population, it is important that aPL-related pregnancy losses occurring before the 10th week of gestation are separated from those occurring after that period. Furthermore, the obstetric APS criteria (further discussed) rightly recognize that a preterm live-birth accompanied by severe preeclampsia or severe placental insufficiency is comparable with a loss late in pregnancy.

The original criteria for the APS were formulated at workshop in Sapporo,Japan in 1998 [7], an international consensus meeting formulated preliminary classification criteria for definite APS and those criteria where revised in 2006 in Sydney , Australia, now known as Sydney classification. [8]. These criteria that include clinical as well as laboratory parameters will be discussed later on in this paper.

aPL and Adverse Pregnancy Outcome.

During the last two decades, several studies have explored the association between aPL and adverse pregnancy outcomes which include maternal effects:

- Venous thromboembolism, including deep vein thrombosis, pulmonary embolism, and cerebral vein thrombosis, Severe preeclampsia, Arterial thrombosis (peripheral, cerebral)

Placental and fetal abnormalities include:

- Thrombosis and infarcts, Abruptio placenta, Recurrent miscarriage, Fetal growth restriction .

aPL are associated with a wide spectrum of adverse pregnancy outcome at all gestational ages—from first trimester miscarriage, through second trimester pregnancy loss, pre-eclampsia, intrauterine growth restriction and preterm labour. Defective embryonic implantation is the unifying feature of all these conditions.

Implantation is a continuous process involving progressive stages—apposition followed by adhesion of the blastocyst to the uterine decidua, and trophoblast invasion of the decidua and maternal uterine spiral arteries. The implantation process starts shortly after conception and continues through the early second trimester of pregnancy. Recent evidence suggests that aPL adversely effect this process. [10]

Maternal risk factors for pregnancy complications are including prior thrombosis, prior pregnancy loss, present organ damage (cardiac, pulmonary and renal), presence of clinically evident SLE and maternal age.

Each factor adds to the risk in addition to the presence of antiphospholipid antibodies alone. Maternal risks include thromboembolism, in addition to complications generally not associated with antiphospholipid antibodies. Also, APS patients are more prone to early-onset, severe pre-eclampsia and hemolysis, elevated liver enzymes, low platelets (HELLP) syndrome. These complications can occur as early as 15– 20 weeks' gestation and might progress rapidly, often necessitating termination of the pregnancy.

Those complications can be differentiated from the symptoms of active SLE, but differential diagnosis can be difficult. Pre-eclampsia and HELLP syndrome can worsen for up to 3 weeks postpartum. On other hand, fetal thrombosis rarely occurs; more often, fetal injury is a result to placental insufficiency, restricted growth (usually first noted after 20 weeks), reduction of amniotic fluid, small placental size and progressive nutrient starvation of the fetus, leading to death. Pathologic studies of affected placentae have demonstrated a variety of vascular and inflammatory changes.

These placentae show a quantitative increase in the levels of IgG and β2GPI compared with control placentae, in addition to co localization of both of these factors with placental anti coagulant protein I.

Infants born to women with APS generally grow normally after birth, but a mild verbal processing difficulty can occur in male children of SLE patients. [11,12]

In order that any therapeutic intervention will be effective it would appear that treatment needs to be applied early in pregnancy, if not before, and it therefore follows that an important part of obstetric management depends on the identification of women at risk of aPL-associated pregnancy complications before they conceive.

Before the introduction of heparin therapy for management of pregnant patients with APS, the fetal loss rate was more than 50%; currently, it is less than 20%.[12]

Pathophysiologic mechanisms of miscarriage in antiphospholipid syndrome.

Several mechanisms have been developed to explain the clinical manifestations associated with antiphospholipid antibodies and their effect on recurrent abortions.

The early hypothesis were concentrating on prothrombotic effect . aPL antibodies have been shown to cause endothelial cell and monocyte activation, leading to a prothrombotic phenotype, which is followed by the expression of adhesion molecules and tissue factors. In order to activate these endothelial and monocytic cells and cause thrombosis, antiphospholipid-antibody–β2GPI complexes interact with cell-surface receptors to induce a signaling cascade.

Also another components of coagulation cascade were implicated .Platelets are also prone to aggregate after exposure to antiphospholipid antibodies. β2GPI complexes bound to antiphospholipid antibodies on platelets interact with the apolipoprotein E receptor and trigger the activation and release of thromboxane, which facilitates platelet aggregation.

It was also shown that aPL induce thrombosis in the uteroplacental circulation and especially spiral arteries. This is mediated by interference with the annexin A5

anticoagulant shield on phospholipid surfaces of trophoblasts and impairment of both intrinsic and extrinsic fibrinolysis.

Recently it was shown that aPL alter the maturation and invasiveness of trophoblast cells *in vitro*. **Table 2** summarize effect of aPL on throphoblast in various researches. Those results suggests that the antibodies cause defective implantation and that thrombosis is not the only explanation for complications of pregnancy in patients with APS. The theory pregnancy complications have multifactorial cause is also supported by the observation that therapies for pregnant women with APS aimed at preventing thrombosis are only partly successful at preventing pregnancy loss.

The new proposed mechanism of inflammatory involvement in pathogenesis of aPL associated pregnancy loss.

Intact complement regulation seems to be essential for maintenance of normal pregnancies. Complement activation promotes recruitment and activation of inflammatory cells. The classical pathway is activated when antibodies bind to antigen and unleash potent effectors associated with humoral responses in immune-mediated tissue damage.

An approach to further define the pathogenesis is to use the antigen binding domain of antiphospholipid antibodies as a means of localizing the pathogenic antibodies. This domain can activate the complement cascade or bind to Fcγ receptors, or both, and thereby trigger activation of the effectors of injury, leukocytes and platelets. Findings from animal models of antiphospholipid antibody- induced pregnancy loss and increased injury-induced thrombosis argue that complement factors C3 and C5 are essential proximal mediators of tissue injury. Some studies suggest that uncontrolled activation of the complement pathway leads to pregnancy failure, even without antiphospholipid antibodies. It means that if antiphospholipid antibodies bind to trophoblasts, exaggerated complement activation overwhelms the inhibitory capacity of local complement regulatory proteins, thereby enabling the complement cascade to proceed.[12,22]

This process leads to recruitment and stimulation of inflammatory cells and injury to the developing fetal–placental unit (**Figure 1**).

Identification women at risk and classification according to previous patient's history.

Lupus anticoagulant (LA) and anticardiolipin antibodies (aCL) are the two phospholipid antibodies most strongly associated with pregnancy complications.

In cases complicated by antiphospholipid syndrome (APS), the patient may be at risk of both recurrent thromboses and abortions. Therefore preconception evaluation is recommended.

It is advisable that this clinical evaluation is performed by a joint team of obstetricians and rheumatologists or internists. Although pharmacological agents are the mainstay of treatment, close obstetric monitoring is mandatory, in order to avoid obstetric complications and determine the optimal time of delivery. The preconception clinical evaluation of women with APS is necessary to focus on the different clinical aspects of the disease. The clinical aspects of APS can differ from patient to patient. Patients with classical APS can have a history of thromboses and/or pregnancy loss and both these aspects can have different presentations.

Additional possibilities include persistently positive antiphospholipid antibodies (aPL) in patients without the clinical features of APS or in patients with primary infertility. Therefore, it may be necessary to identify and classify patients before conception. Here is example of such classification that may modify treatment.[11, 13]

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Clinical classification of patients at preconception evaluation (Adapted from *A Tincani et al).*

(A) Previous thrombosis

- Arterial
- Venous
- Small vessel disease/microangiopathy

(B) Previous pregnancy loss or complication

- Early losses (three or more prior to 10 weeks of gestation)
- Late losses (one or more after 10 weeks of gestation)
- Premature births (one or more prior to 34 weeks of gestation) as a consequence of severe preeclampsia or placental insuficiency
- Growth restriction and placental abruption

(C) Previous thrombosis and pregnancy loss or complication

(D) Persistently positive antiphospholipid antibodies

- Associated with SLE or other autoimmune disorders
- In apparently healthy women

(E) Persistently positive antiphospholipid antibodies in women undergoing in vitro fertilization

- Associated with SLE or other autoimmune disorders
- In apparently healthy women

==========

During the preconception evaluation there should be considered that genetic thrombophilic risk factors should be investigated, especially if the patients reported on a history of thrombosis. The most frequently considered factors are: hyperhomocysteinaemia, ATIII, protein C and protein S deficiencies and the factor V Leiden and factor II (G20210A) mutations.

1. Clinical and serological diagnosis of antiphospholipid syndrome as a secondary prevention of recurrent abortions

Recurrent abortions affects around 1% of fertile couples. Although in majority of cases the ethiology remains unknown, antiphospholipid syndrome is manageable cause .

As it was already mentioned before - for any therapeutic intervention to be successful it would appear that treatment needs to be applied early in pregnancy, if not before, and it therefore depends on the identification of women at risk of aPL-associated pregnancy complications before they conceive.

As a result, I think , that basic investigations of a couple presenting with recurrent miscarriage should include obstetric and family history, age, BMI and exposure to toxins, full blood count, antiphospholipid antibodies (lupus anticoagulant and anticardiolipin antibodies), parental karyotype, pelvic ultrasound and/or hysterosalpingogram.

And as concerning therapy – before the introduction of heparin therapy for management of pregnant patients with APS, the fetal loss rate was more than 50%; currently, it is less than 20%.[12]

Therefore both diagnostical and therapeutic methods should be considered in prevention of recurrent abortions.

1.1 Clinical diagnostic criteria

As it was discussed earlier in introduction, since year 1999 the formulation of the international preliminary classification Sapporo criteria for antiphospholipid syndrome (APS), an additional work in basic research and studies on laboratory and clinical manifestations of APS has appeared. In 2006 the evidence was also reviewed and graded by Sydney classification .The Sapporo classification divided

the APS criteria into clinical and laboratory; this categorization was maintained in the 2006 revision.

Revised classification criteria for the antiphospholipid syndrome summarized in **Table 2.**

Clinical obstetric criteria for the diagnosis of antiphospholipid syndrome presented in **Table 1.**

1.2 Serologic diagnostic criteria

Here are laboratory criteria that are included into revised classification criteria for the antiphospholipid syndrome (**Table 2**) [8]

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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 , on two or more occasions, at least 12 weeks apart, measured by a standardized ELISA.

3. Anti-b2 glycoprotein-I antibody of IgG and/or IgM isotype in serum or plasma , present on two or more occasions, at least 12 weeks apart, measured by a standardized ELISA.

Some of the laboratory features were not included in the revised classification criteria for APS will be discussed also.

These include:

(1) IgA aCL,

(2) IgA anti-b2GPI,

(3) antiphosphatidylserine antibodies (aPS),

(4) antiphosphatidylethanolamine (aPE) antibodies,

(5) antibodies against prothrombin alone (aPT-A),

(6) antibodies to the phosphatidylserine–prothrombin (aPS/PT) complex.

Some of the antibodies above are frequent but not specific in patients with APS. The classification committee considered that inclusion of this antibodies into criteria for APS may decrease diagnostic specificity, even though their association with APS is recognized.[8]

Lupus anticoagulant strongly associated with the antiphospholipid antibody syndrome. Lupus anticoagulants role is described as the tendency of antiphospholipid antibodies to prolong the clotting times, especially in phospholipid rich clotting testing such as the dilute Russell's viper venom time.

There are different recommendations that can be given on the assays of choice for LA testing. Both activated partial thromboplastin time (APTT)-based assays and dilute Russell's viper venom time (dRVVT) are suitable for LA.

One positive test suffices for LA positivity; as no single test is 100% sensitive for LA, it is advised to use two ormore tests with different assay principles before the presence of LA is excluded.
Unless one uses an LA test system that includes a heparin neutralizer (most of the commercial dRVVT-assays), the thrombin time should always be measured to exclude unforeseen presence of unfractioned heparin. If the patient is on oral anticoagulants, measurement of LA is better postponed , or patient samples be diluted 1 : 2 with normal plasma before the test is performed, provided that international normalized ratio (INR) is <3.5. When INR is >3.5, the LA testing is unworkable**.[7]**

Anti-b2GPI . By majority of experts agreed that IgG and IgM antib2GPI should be included as part of the modified Sapporo criteria. Anti-b2GPI antibodies are an independent risk factor for thrombosis and pregnancy complications. The anti-b2GPI assay shows higher specificity than aCL for APS diagnosis.
In 3–10% of APS patients, anti-b2GPImay be the only test positive. The association of anti-b2GPI with pre-eclampsia and/or eclampsia in unselected

pregnant women who tested negative for aCL implies that the inclusion of anti-b2GPI may also help clarify this pregnancy morbidity.

High titers of anti-b2GPI antibodies are associated with high risk of thrombosis, but it is difficult to define boundaries for medium and high titers at this stage. Until an international consensus is reached, this committee proposes a threshold for positive anti-b2GPI antibodies >99th percentile of controls. [7]

Anticardiolipin assay. The classification committee recommends that aCL continue to be a laboratory criterion for APS. Also some experts suggest that LAG and anti-b2GPI are sufficient for laboratory criteria.

The original study showed that patients with elevated aPL levels at their initial prenatal visit had an increase in fetal loss but no increase in maternal pregnancy complications.[20]

The IgA aCL are usually detected together with either IgG and/ or IgM isotypes in patients with APS, and agreement among patients grouped according to aCL titers for IgA seems lower than those for the other isotypes. Specificity and standardization considerations for the other aCL isotypes apply also to the IgA aCL assay.The committee consents that IgA aCL cannot be considered as a laboratory criterion for APS.

IgA anti-b2GPI and other ELISAs for aPL detection.

Data are inadequate for establishing IgA anti-b2GPI as an independent risk factor for APS in the absence of other antib2GPI isotypes. IgA anti-b2GPI are the most frequently detected antibodies in patients in specific ethnic groups. A significant proportion of IgA anti-b2GPI-positive tests has no apparent association with any clinical manifestation of APS.

Definite guidelines how to perform the test, units of measurement and control materials do not exist. The committee concludes that it is premature to recommend that tests for an aPL other than IgG and IgM anti-b2GPI be included in the Sydney classification criteria.[7]

Antiprothrombin antibodies

Antiprothrombin antibodies detected by ELISA are a heterogeneous population including antibodies against prothrombin alone (aPT-A) and antibodies to the phosphatidylserine– prothrombin complex (aPS/PT). Data on the clinical associations of aPT-A are contradictory, and they imply low specificity of these antibodies for APS diagnosis .Both the sensitivity and specificity of aPS/PT are higher than those for aPT-A, whereas 95% of patients with aPS/PT are also LA positive. This committee considers that the inclusion of antiprothrombin antibodies in the classification criteria for APS is premature.[7,21]

2. Therapy modalities as a prevention of recurrent abortions in patients with antiphospholipid syndrome

Combined unfractionated heparin and low-dose aspirin regimens are thought to reduce the risk of spontaneous pregnancy loss by 54%, resulting in a live-birth rate of 70–80%.

Successful treatment, defined as fetal survival, is also the result of careful obstetric monitoring, early delivery and skilled neonatal care. Despite high fetal survival rates, however, prematurity and low fetal birth weight are notable risks.

There are several evidence- based studies that reach consensus on the therapy of patients with APS during pregnancy and abortion prevention, which explore different clinical situations. Clinical cases classified according to previous patient's history as it was shown previously in introduction.

Treatment or prevention of recurrences

(1) Patients with previous arterial or venous thrombosis

In this group of patients, a consensus was reached on the administration of heparin and low dose aspirin (LDA) in pregnancy. However, two experts pointed out that no evidence-based medicine supports the use of LDA. One expert said that a patient who was given LDA for a previous arterial thrombosis would continue with LDA alone and another patient given warfarin for a deep vein thrombosis (DVT) would be switched to heparin.

The general opinion was to treat with low molecular weight heparin (LMWH), leaving unfractioned heparin (UH) for emergency situations such as the delivery day.

There were concerns about the high costs of LMWH and that LMWH is not approved for use in pregnancy in some countries. The LMWHs used included enoxaparin (1mg/kg or 30–80mg twice daily), dalteparin (5000 IU twice daily) and nadroparin calcium (0.4ml twice daily). The dosage should be adjusted according to the body weight (e.g., enoxaparin 1mg/kg twice daily) and previous history of thrombosis (with a higher dosage for severe arterial thrombosis). Two centres suggested starting with one injection per day until the 16th week and every 12 hours thereafter. When UH is used during pregnancy, subcutaneous injections are also given three times a day to achieve anticoagulation according to mid-interval heparin levels.

This treatment is generally started as soon as pregnancy is diagnosed (bHCG positive) in order to avoid use for too long, but definitely before the seventh week of gestation. Warfarin is not used at this early stage due to its teratogenicity. If menstruation is irregular, bHCG may be measured monthly to avoid missing the onset of pregnancy.

The time to stop the treatment is more controversial. Some centres advised stopping therapy on the day of delivery or, if possible, six to 24 hours before delivery.

Because of the possible effect of LDA on epidural anaesthesia, LDA is discontinued five to six weeks before the potential delivery day in some countries.

Some experts switch the therapy back to oral anticoagulant six to eight hours after delivery, whereas others continue with LMWH for a period ranging from one to 12 weeks after delivery.

The same treatment is generally administered to pregnant patients with a previous arterial or venous thrombosis, although one expert advised adding aspirin only in patients with arterial thrombosis. Patients with thromboses on heparin, severe thromboembolism or thrombotic strokes are considered for treatment with warfarin from 14 to 34 weeks of gestation. In one centre all patients with previous thromboses are treated with warfarin from 14 to 36 weeks.

(2) Patients with previous pregnancy losses or complications

A general consensus was reached on the use of LDA and LMWH (or UH because of its low cost) in this group of patients. Only two centres recommended a different approach: in one centre, LDA was used alone and heparin only used for patients with losses only were treated with LDA alone and those with late losses with LMWH and LDA.

The dosage of LMWH is generally lower than that administered to group A patients: enoxaparin 1mg/kg/ day or 40–80mg/day; dalteparin 5000 IU; nadroparin calcium 0.4ml (3800 IU). If UH is used, injections are administered twice daily at a dosage of 15000–20000 units per day.

Heparin is started when the pregnancy test is positive; however, most patients may already be receiving aspirin from the preconceptional clinical evaluation.

Treatment should be discontinued on the day of delivery, or if possible, six to 24 hours before delivery. Heparin is generally resumed again six to eight hours after delivery for an average period of six to eight weeks. In two centres no prophylactic treatment is used during the puerperium, while in one centre warfarin is prescribed for six to eight weeks post partum.

The majority of the centres make no distinction between early and late pregnancy losses, however one centre treats women with early losses with LDA and late losses with LDA and LMWH, and one other centre uses higher heparin doses in the prophylaxis of patients with late losses.

In some patients with early pregnancy losses and aPL an abnormal embryonic or parental karyotype may be found. In these patients LDA is generally used without heparin, but the role of therapy should be discussed with the patients.

(3) Patients with previous thrombosis and pregnancy loss or complications

No substantial differences were reported regarding the treatment of patients with a history of both thrombosis and pregnancy loss. These patients generally receive the same therapy as the group 1 patients. Some centres suggest increasing the dosage of LMWH to effective anticoagulant levels in cases of venous thrombosis and a late pregnancy loss.

(4) Patients with persistently positive antiphospholipid antibodies

Majority of experts use aspirin when these patients are pregnant, although this treatment is not evidence-based; other experts prefer not to prescribe any drug. In one centre there is an ongoing case–control study comparing LDA to placebo. If the patient has persistently positive aPL tests and SLE, treatment is usually the same (LDA or no drug therapy). However, the drugs required to control lupus will be continued (corticosteroids, hydroxychloroquine or azathioprine).

(5) Persistently positive antiphospholipid antibodies in women undergoing in vitro fertilization

Patients with primary infertility and antiphospholipid antibodies (without thrombotic episodes) can be treated . Most of the experts agreed that heparin (LMWH or UH) is indicated during the procedures associated with *in vitro* fertilization due to the high oestrogen levels following gonadotrophin stimulation. However, heparin therapy was discontinued 24 hours prior to ovum pick up, so as not to cause haemorrhage during this procedure. On the contrary, there was no consensus regarding treatment of pregnancy following IVF: some centres give no medication, whereas others treat with LDA throughout pregnancy and others add LMWH for at least 10 weeks in order to prevent thrombosis. [9, 13]

The risk of fetal loss in asymptomatic pregnant carriers of antiphospholipid antibodies who have not had prior pregnancy losses is unknown but is estimated to be in sufficiently high to justify treatment with heparin. Because they have an increased risk of thromboembolism, patients who are receiving anticoagulants for fetal protection continue such treatment for 3 months postpartum. Although it is recommended that this substitution occurs when pregnancy is first attempted, failure to conceive will result in potentially prolonged therapy.

Most guidelines recommend now use low-molecular weight heparin because formulations are as effective as, and less dangerous than, unfractionated heparin preparations, and their use has improved management. Enoxaparin is given twice daily at doses of 0.5 mg/kg body weight (prophylactic dose) or 1.0 mg/kg body weight (therapeutic dose).

The activated partial thromboplastin time (a measure of coagulation) is conventionally used to evaluate the treatment effect of heparin therapy. Because lupus anticoagulant causes a prolonged activated partial thromboplastin time, this measurement cannot be used in patients with lupus anticoagulant. Instead—if necessary— the heparin dose can be monitored by measurement of the activity of antifactor Xa.

If lupus anticoagulant is not present and the baseline clotting time is normal, the activated partial thromboplastin time during therapy should be kept at 2.0–3.0 times that of normal at peak and at least 1.5 times that of normal immediately before the next dose. Women with APS-associated fetal loss should be prescribed low-molecular-weight heparin at the doses described above, with concomitant low-dose aspirin (50–100 mg daily). Low-dose aspirin therapy theoretically has value because of its protective effects on placental function.[12]

Figure 3 summarize possibilities of treatment that were discussed earlier in form of algorithm.

Heparin mechanism of action.

As it was discussed earlier placentae from patients with APS treated with antiphospholipid antibodies show signs of inflammation.

Potentially, the anti-inflammatory properties of heparin could contribute to the efficacy of therapy. Heparins affect inflammatory cell function by preventing leukocyte adhesion, infiltration and tissue damage (**Figure 2**).

Unfractionated heparin blocks cell adhesion initiated by P-selectin and L-selectin *in vivo* and *in vitro*. Low-molecular-weight heparin has been shown to inhibit leukocyte rolling, adhesion to vascular endothelial cells and extra vascular accumulation. Heparin have also been shown to increase the cellular motility and invasiveness of an extravillous trophoblast cell line and recruitment and adhesion of leukocytes (**Figure 2**).

Other possible mechanism suggest that heparin are involved in the adhesion of the blastocyst to the endometrial epithelium, and its subsequent invasion, and that they have the capacity to interfere with blastocyst–uterine cross talk during implantation. Additionally, heparin therapies could minimize the damage caused by antiphospholipid antibodies by altering the binding of antiphospholipid antibodies to phospholipids on target cells and/or limiting the binding of β2GPI to phospholipids, which would prevent targeting by, and deposition of, anti-β2GPI antibodies. Finally, heparin has been shown to directly reduce the generation of inflammatory mediators.

Heparin was shown to have anticomplement effects and subsequent studies have identified several possible mechanisms for this activity (**Figure 2**). Heparin can inhibit complement activation at various points in the classical, alternative and terminal pathways, including inhibiting C1q binding to immune complexes and interfering with interactions of C4 with C1s and C2. In addition, heparin might block the formation of C3 amplification convertase by the alternative pathway and inhibit the formation of the membrane attack complex.[12 , 13]

IVIG and immunomodulation therapies.

There are only few cases of intravenous immunoglobulin (IVIG) use. Mostly there is no experience. Those who have used this treatment consider it as a second line treatment when other more standard therapies have failed, or when severe thrombocytopenia is a complicating feature.

The mechanisms of action of IVIG include solubilizing immune complexes and antiidiotypic downregulation of autoantibody production.

However, an additional mechanism is that IVIG causes down regulation of systemic natural killer (NK) cells, elevated levels of which are associated with recurrent miscarriage. It is theorized that NK cell activity at the implantation site is counteracted by IVIG, thereby lowering the risk of miscarriage. However, a recent study of IVIG therapy for women with unexplained recurrent miscarriage failed to demonstrate a clinically useful benefit from this treatment .[11]

When IVIG is used for pregnant patients with APS, the suggested dosage is 0.4mg/kg in repeated infusions for two to five days each month.[13]

Prednisone and other immunomodulating therapies are seldom prescribed for pregnant women with APS, but prednisone is appropriate for clinically active SLE, if present. A small study of APS patients with and without SLE who were treated with 40 mg prednisone daily or heparin (10,000 IU twice daily at 6–8 weeks, reduced to 2000 IU twice daily to attain normal activated partial thromboplastin time at mid trimester), both with concomitant low-dose aspirin (81 mg), demonstrated equally high rates of live births in both treatment groups. Yet, maternal complications were greater in the prednisone-treated group. The doses used ranged widely, from 0.4 g/kg body weight per trimester to 2.0 g/kg body weight monthly.[12]

Treatment of thrombocytopenia.

Falling platelet counts or thrombocytopenia might be caused by pre-eclampsia, HELLP syndrome, placental insufficiency, worsening maternal APS, active SLE or idiopathic thrombocytopenic purpura. Late-onset thrombocytopenia, a common phenomenon during normal pregnancies, occurs frequently in pregnant patients

with APS and could signal an increased risk of fetal injury. Treatment for platelet counts more than 50,000 (50 × 109/l) is usually unnecessary. If required, the management of severe thrombocytopenia in pregnant patients with APS consists of control of blood pressure, administration of intravenous immunoglobulin and early delivery.[13]

Maternal and fetal monitoring.

Women receiving heparin should have regular platelet counts performed in order to detect the rare occurrence of heparin induced thrombocytopenia. Monitoring aPL titres during pregnancy appears to be of no benefit because of the marked intra-individual variation in test results.

As in all cases of recurrent miscarriage, success is largely determined by maternal age and the number of previous pregnancy losses.

Fetal welfare is monitored with ultrasound assessments of growth and Doppler studies of the uterine and umbilical circulations. Abnormal Doppler studies are predictive of pregnancies at increased risk for the development of pre-eclampsia and intrauterine growth restriction. At present, ultrasound assessment of fetal welfare aids solely in the timing of delivery of the fetus. However, the potential exists for women with pregnancies identified to be at increased risk to be recruited to future therapeutic intervention studies.[9,11]

Potential therapy risks.

I tried to explore the potential danger of using present type of therapy for mother and fetus. Comparing benefit of therapy and adverse effect:

The possible relationship between aspirin in early pregnancy and congenital defects remains controversial. Initial reports such as the Collaborative Perinatal Project found no evidence of a teratogenic effect. The Food and Drug Administration (FDA) surveillance study involving 1709 newborns exposed to aspirin during the first trimester presented similar conclusions. A more recent meta-analysis has found that the overall risk of congenital malformations in offspring of women exposed to aspirin in early pregnancy is not significantly

higher than that in control subjects . However, a significant increase in the risk of fetal gastroschisis (odds ratio 2.37, 95%) was found.

There is no reported fetal side-effect of heparin use during pregnancy, but osteopenia has been a major concern of long-term heparin therapy, in particular with unfractionated heparin (UFH). In women with RM, a small decrease of 3.7% of lumbar spine and 0.9% of the neck of femur bone mineral density (BMD) has been reported in one study using both LMWH and UFH . There was no significant difference in BMD changes, in this uncontrolled study, between the two heparin preparations. A more recent prospective, controlled study has shown that bone loss associated with the use of long-term LMWH for RM and thrombophilia is not significantly different from physiological losses during pregnancy . Overall, the decrease in BMD seems to be similar in heparin-treated and untreated pregnant women . Fondaparinux sodium, a new indirect activated factor VII inhibitor, does not have a negative effect on BMD and could therefore be a safe and effective alternative to UFH and LMWH. Treatment-related thrombocytopenia was not reported in a recent systematic review of 64 studies of LMWH use in pregnancy.[16,17,18]

Conclusion

Presented data show the big progress that was done in a past decade in diagnosis and treatment of recurrent abortions in antiphopholipid syndrome patients. Nevertheless it also reveals some uncertanties in diagnosis and difficulties in treatment. It largely results from insufficient knowledge of ethiology, mechanisms of disease progression and development of new diagnostic methods. It is well established that women with a history recurrent miscarriage have an increased risk of recurrence in subsequent pregnancies. The rate of recurrence o may be as high as 46% with a history of 2 or more adverse outcomes.

Based on these findings, some it should be recommended to screen for aPL in women who have had adverse pregnancy outcomes and prophylactic therapy in subsequent pregnancies when the test is positive.

Serologic criteria are still debatable. The question of aCL isotype, IgG or IgM, and titre that confers a significant risk for pregnancy loss is still present. This is in part due to (a) the widespread inter-laboratory variation in the detection of aCL (b) the heterogeneity of aPL and (c) the different clinical criteria used in the recruitment of patients for studies.

The challenge for the future is to better characterize and discriminate between those aPL that do cause recurrent abortions and those that do not.

Summary.

Recurrent abortions prevalent in around 1% of couples. Although in majority of cases the ethiology remains unknown, antiphospholipid syndrome is cause that can be prevented.

The antiphospholipid antibody syndrome (APS) is characterized by arterial and venous thrombosis and pregnancy complications in association with antiphospholipid (aPL) antibodies. In addition to recurrent abortions and fetal death, pregnancy complications in women with APS include preeclampsia, placental insufficiency, and fetal growth restriction . The pathogenic mechanisms that lead to injury *in vivo* are incompletely understood and therapy for pregnant women with APS, currently aimed at preventing thrombosis, is only partially successful in averting pregnancy loss.

As it was already mentioned before - for any therapeutic intervention to be successful it would appear that treatment needs to be applied early in pregnancy, if not before, and it therefore depends on the identification of women at risk of aPL-associated pregnancy complications before they conceive.

Before the introduction of heparin therapy for management of pregnant patients with APS, the fetal loss rate was more than 50%; currently, it is less than 20%.

Therefore both diagnostical and therapeutic methods should be considered in prevention of recurrent abortions.

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Attachment of pictures and tables .

Table1 . ***Clinical obstetric criteria for the diagnosis of antiphospholipid syndrome. **

1	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*
2	One or more premature births of a morphologically normal neonate at or before the 34th week of gestation because of severe pre-eclampsia or eclampsia, or severe placental insufficiency *or*
3	Three or more unexplained consecutive spontaneous abortions before the 10th week of gestation, with maternal anatomic or hormonal abnormalities and paternal and maternal chromosome causes excluded

*Adopted from : Raj Rai Department of Reproductive Science and Medicine, Imperial College School of Medicine at St Mary's, Mint Wing, London, UK. 2000 Academic Press

Table 2

Summary of antiphospholipid antibody effects on trophoblast function

Antibody	Cell type	Binding	Trophoblast proliferation	HCG and HPL secretion	Trophoblast invasiveness	Trophoblast fusion	Reference
Polyclonal aPL (IgG)	Primary trophoblast cells	++		Reduced by 40%	Completely blocks	Completely blocks	Di Simone Et al. (1999)
Anti-β2-glycoprotein I	Choriocarcinoma cells	++	Completely blocks				Chamley et al. (1998)
Antiphosphatidylserine	Choriocarcinoma cells	++		Reduced by 40%	Completely blocks	Completely blocks	Rote et al. (1995, 1998)
Antiphosphatidylserine	Primary trophoblast cells	++		Reduced by 50%	Completely blocks		Katsuragawa et al. (1997)

HCG = human chorionic gonadotrophin;

HPL = human placental lactogen

Table 3

Antiphospholipid antibody 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 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 pre-eclampsia defined according to standard definitions [11], 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.

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 (LA) present in plasma, on two or more occasions at least 12 weeks apart, detected according to the guidelines of the International Society on Thrombosis and Haemostasis (Scientific Subcommittee on LAs/phospholipid-dependent antibodies) [82,83].

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 [100,129,130].

3. Anti-β_2 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, according to recommended procedures [112].

Figure 1

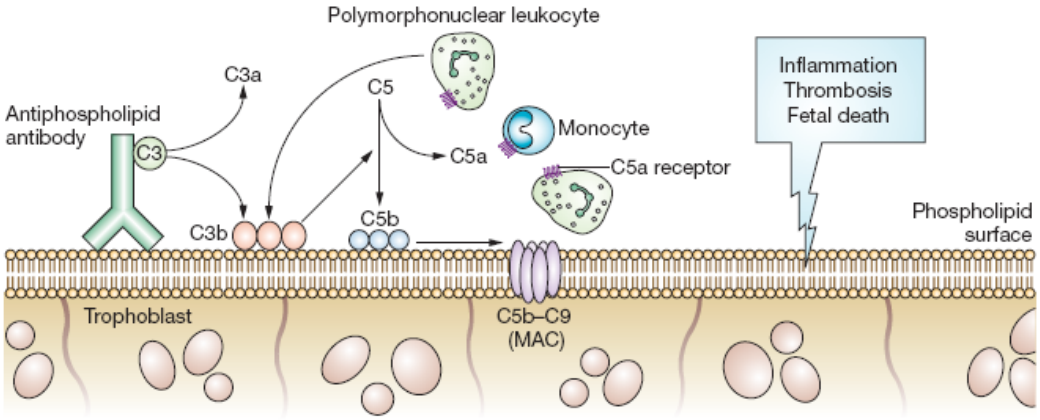

Figure 1 Proposed mechanism for the pathogenic effects of antiphospholipid antibodies on tissue injury. Antiphospholipid antibodies are preferentially targeted to the trophoblast cells of the placenta where they activate complement through the classical pathway. The complement cascade is initiated; C3 and, subsequently, C5 are activated. C5a is generated, which attracts and activates neutrophils and monocytes and stimulates the release of inflammatory mediators, including reactive oxidants, proteolytic enzymes, chemokines and cytokines. Complement activation is amplified by the alternative pathway, which results in further influx of inflammatory cells and, ultimately, fetal injury. Depending on the extent of damage either death *in utero* or fetal growth restriction ensues. Abbreviation: MAC, membrane attack complex.

Figure 2

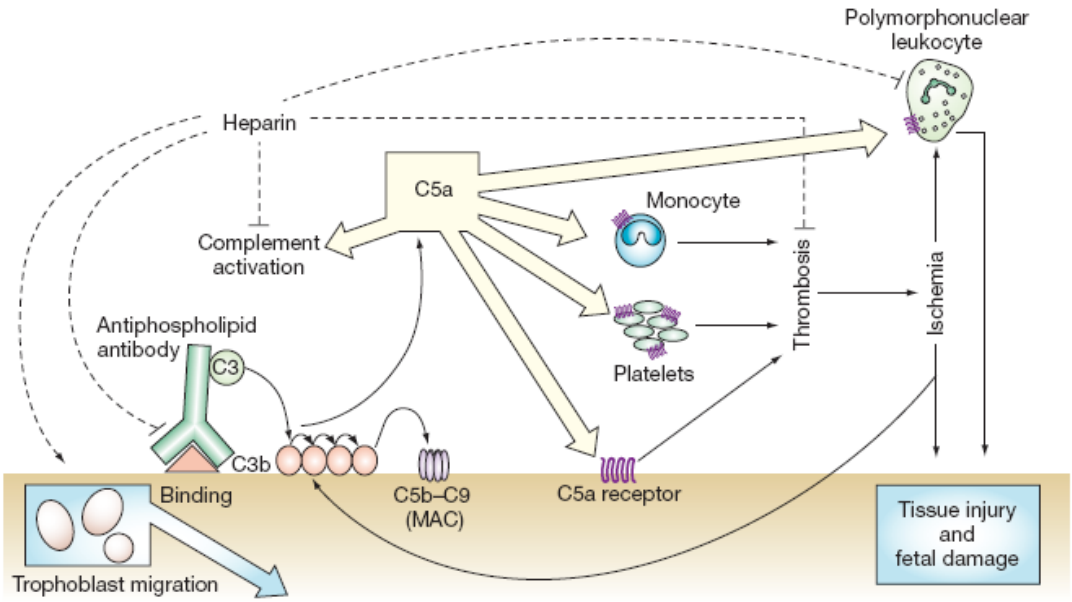

Figure 2 Potential mechanisms by which heparin prevents fetal and placental injury by antiphospholipid antibodies. In addition to inhibiting coagulation, heparins have anti-inflammatory effects. They prevent leukocyte adhesion to vascular endothelial cells and transmigration and block activation of complement at multiple levels of the cascade. Heparins might also limit antiphospholipid-antibody-mediated damage because they limit antibody targeting to trophoblasts and enhance trophoblast invasiveness. Abbreviation: MAC, membrane attack complex.

Figure 3. ***Algorithm for pharmacological treatment of women with LAC, anticardiolipin antibodies, or a combination of these during pregnancy and the postpartum period.***[9]