The physiologic anticoagulant and anti-inflammatory role of heparins and their utility in the prevention of pregnancy complications

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Summary
Accumulating evidence supports the concept of increased thrombin generation, placental vascular lesions, and inflammation as crucial points in the development of the great obstetrical syndromes [pre-eclampsia, intrauterine growth restriction (IUGR), preterm labor (PTL), preterm prelabor rupture of membranes (PROM), fetal demise and recurrent abortions]. In light of this, the role of heparins for primary or secondary prevention of these syndromes becomes more apparent, mainly due to the antithrombotic and anti-inflammatory effects of heparins. There is agreement regarding the use of heparin in the prevention of gestational complications in patients with antiphospholipid syndrome, while its use for other obstetrical complications is under debate. In the present review we will describe the physiologic role of heparins on coagulation and inflammation and we will discuss current evidence regarding the use of heparins for the prevention/treatment of obstetrical syndromes.

Keywords
Antiphospholipid syndrome, inflammation, preterm labour, recurrent pregnancy loss, thrombin generation

Introduction
The understanding that thrombosis is a mechanism of disease in many of the great obstetrical syndromes (1) [preeclampsia, intrauterine growth restriction (IUGR), preterm labor (PTL), preterm prelabor rupture of membranes (PROM), fetal demise and recurrent abortions], along with the reports (2, 3) regarding the role of acquired and inherited thrombophilia in these syndromes, led to the increased use of heparins [unfractionated (UFH) and low-molecular-weight heparins (LMWH)] in obstetrics. Nevertheless, recent evidence has questioned this practice.

In this review we will describe the physiologic role of heparins, their impact on coagulation and inflammation and their use for the prevention of pregnancy complications. To address this task we have done a comprehensive review of all the randomised clinical trials and systematic reviews published in the English literature from 1966 until present. Among them, those comparing treatment with heparin vs placebo or vs other medication were considered.

The physiological role of heparin
Heparin is one of the oldest drugs currently in widespread clinical use. Its discovery in 1916 predates the establishment of the Food and Drug Administration of the United States, although it did not enter clinical trials until 1935. It was originally isolated from canine liver cells, hence its name (hepar or “ηπαρ” is Greek for “liver”).

Although it is used principally in medicine for anticoagulation, its true physiological role in the body remains unclear because blood anticoagulation is achieved mostly by heparan sulfate proteoglycans derived from endothelial cells (4). Heparin is usually stored within the mast cell secretory granules and is released only into the vasculature at sites of tissue injury. It has been proposed that, in addition to its anticoagulant properties, heparin may play a role in the defense against invading bacteria and other foreign materials (5).

Heparin is a glycosaminoglycan composed of chains of alternating d-glucosamine and uronic acid residues. It’s major anticoagulant effect is attributed to a unique pentasaccharide structure (GlcNAc/NS(6S)-GlcA-GlcNS(3S,6S)-IdoA(2S)-GlcNS(6S), that has a high binding affinity sequence to antithrombin III (AT-III). Of interest, in vitro studies suggest that this structure is present only in about one-third of heparin molecules (6).

What is the mechanism of the anticoagulant activity of heparins?
The interaction between heparin and AT-III mediates the majority of the anticoagulant effects of the former. Their binding produces a
The anti-inflammatory effects of heparins are derived from several mechanisms: 1) the molecular structure of these molecules is such that upon their binding to endothelial cells of blood vessels, a negatively charged surface that faces the vessel lumen is created. These negatively charged molecules repulse negatively charged leukocytes and prevent their adhesion to the endothelium (heparin activity against thrombin is size-dependent, and the ternary complex (Figure 2) requires at least 18 saccharide units for efficient formation and thrombin inactivation (8, 9).

In contrast, the effect of heparin on the inhibition of factor Xa by AT-III is dependent only on the conformational change of this molecule at the heparin-binding site. This has therapeutic implications and led to the development of a new generation of heparin-derived anticoagulants, including LMWH and fondaparinux. LMWHs are obtained as fragments of UFH as a result of enzymatic or chemical depolymerisation, yielding molecules with a mean weight of 5,000 Dalton (Table 1), while fondaparinux is a synthetic pentasaccharide based on the heparin antithrombin-binding domain (10).

These medications target the anti-FXa activity rather than the antithrombin (IIa) activity of AT-III, aiming to facilitate a more subtle regulation of coagulation with an improved therapeutic index and less side effects. Indeed, each molecule of fondaparinux binds to one molecule of AT-III at a specific site and with very high affinity. The binding is rapid, non-covalent, and reversible. It induces a critical conformational change in AT-III, exposing a loop containing an arginine residue that binds FXa. Exposure of the arginine-containing loop greatly increases the affinity of AT-III for FXa, potentiating the natural inhibitory effect of AT-III against FXa by approximately 300-fold (8, 9).

What is the mechanism of the anti-inflammatory activity of heparins?

The anti-inflammatory effects of heparins are derived from several mechanisms: 1) the molecular structure of these molecules is such that upon their binding to endothelial cells of blood vessels, a negatively charged surface that faces the vessel lumen is created. These negatively charged molecules repulse negatively charged leukocytes and prevent their adhesion to the endothelium (heparan sulfate molecules that are expressed on leukocytes surface are responsible for the negative charge of these cells); 2) heparin is a large molecule that can bind to a substantial number of proteins playing important roles in inflammation, including selectins (L-selectin [11] and P-selectin molecules [12]) and integrins. The B2-integrin adhesion molecule CD11b/CD18, also known as macrophage antigen 1 (MAC1), is a member of a subfamily of related cell-surface glycoproteins that coordinate adhesive functions including leukocyte migration (13). MAC1 is expressed on myeloid cells and binds to molecules such as intercellular adhesion molecule 1 (ICAM1), fibrinogen, iC3b, and FXa. The heparin-MAC1 bond interferes with myeloid cell adhesion and transmigration (14). Heparin also binds to platelet/endothelial cell adhesion molecule 1 (PECAM1), a member of the Ig superfamily, which is expressed on a variety of cells, such as platelets, endothelia, monocytes, neutrophils, T-cell subsets and granulocyte/macrophage precursors. This molecule is involved in homotypic and heterotypic cellular adhesion and plays a role in the transmigration of inflammatory cells through the endothelial wall. Heparin is capable of binding PECAM1 and interfering with its action (15), thus reducing the effectiveness of the inflammatory response.

The anti-inflammatory properties of LMWH have been demonstrated with in vivo models. Indeed, Wang et al. (16) investigated the effects of LMWH on dextran sulfate sodium (DSS)-induced colitis in a mouse model. The authors reported that mice treated with LMWH had a significant decrease in the expression of both IL-1β and IL-10 mRNA, leading to a downregulation of inflammatory cytokine production. Of interest, LMWH also imitates the function of Syndecan-1, a protein that plays an important role in promoting wound repair, maintaining cell morphogenesis, and mediating inflammatory responses (17) by aiding the clearance of pro-inflammatory chemokines. Syndecan-1 expression is inversely correlated to the mRNA expression of IL-1β in the intestinal mucosa of DSS-induced colitis mice, and this may be the explanation for the effect of LMWH. In addition, Li et al. (18) found that treat-
Mastrolia et al. Heparins and pregnancy complications

Unfractionated heparin

Figure 2: Mechanisms of interaction between heparin, antithrombin, thrombin and factor Xa. Heparin binding leads to a conformational change in antithrombin (AT)-III that accelerates up to 1,000-fold its ability to inactivate thrombin (factor IIa) and factor Xa. The acceleration of the inhibitory effect of AT-III necessitates its binding as a complex, with an activate coagulation factor, to the heparin polymer proximally to the pentasaccharide units. However, the mechanisms for heparin effect differ in each coagulation factor: 1) Heparin increases the inhibitory effect of AT-III on thrombin in a size-dependent manner, and at least 18 saccharide units are required in the heparin molecule for an efficient formation of the ternary complex and thrombin inactivation; and 2) In contrast, the effect of heparin on the inhibition of factor Xa by AT-III is dependent only on the conformational change of this molecule at the heparin-binding site. From Quaranta M, et al. Peer J 2015; 3: e691 https://dx.doi.org/10.7717/peerj.691. Reproduced with permission.

The use of heparins in the prevention of pregnancy complications

Pregnancy complications as obstetrical syndromes

The term ‘great obstetrical syndromes’ was coined by Roberto Romero (23) to reframe the concept of obstetrical diseases. This term comprises all obstetrical complications such as preterm labor (PTL), preterm prelabor rapture of membranes (PROM), fetal demise (FD), preeclampsia, and intrauterine growth restriction (IUGR). These syndromes are the clinical endpoint of several underlying mechanisms, including intrauterine or systemic maternal infection/inflammation; uterine ischaemia; placental vascular lesions; abnormal allograft reaction; and others (23). In addition, many of the obstetrical syndromes are associated with an increased generation of thrombin (24, 25). This has led to the perception that pregnant women may benefit from treatment with heparins for the prevention of such complications.

In humans, the anti-inflammatory activity of heparins has been evidenced by small clinical trials in patients suffering from a range of inflammatory diseases (19) including rheumatoid arthritis and bronchial asthma. Remission of disease has been described in nine of ten patients with refractory ulcerative colitis treated with combined heparin and sulphasalazine (19). A subjective improvement of asthma symptoms using intravenous heparin is described (20), while other studies with inhaled heparin demonstrated reduced bronchoconstrictive responses in patients with exercise-induced asthma (21). The clinical rationale for the use of heparins in the treatment of inflammatory diseases may be based on the fact that many of the molecular mechanisms involved in tumour metastasis are also responsible for cell recruitment in inflammation; and heparins has been successful in treating both conditions (22).
The use of heparins in patients with antiphospholipid syndrome

Antiphospholipid antibody (APLA) syndrome was considered until recently to be a prototype of a hypercoagulable state. These patients are at increased risk for thromboembolic disease and pregnancy complications (26). Indeed, approximately 15% to 20% of pregnancies in women with this syndrome are complicated by pregnancy loss after nine weeks (at least 50% of them occur after the 10th week of gestation), placental abruption, pre eclampsia, IU GR and late fetal death (27). In a large, prospective cohort of 1,155 women, a panel of APLA was evaluated in the first trimester to determine their relationship to adverse pregnancy outcomes. The association of antiphosphatidylethanolamine IgG and anticardiolipin IgG (odds ratio [OR] 17.5; 95% confidence interval [CI] 4.7–66.7) or antiphosphatidylethanolamine IgG and lupus anticoagulant (OR 22.2; 95% CI 5.4–90.9) (28) and a positive LAC test had an OR of 3.0 to 4.8 for fetal loss (26). Moreover, the placental findings in these patients included thrombosis and vascular lesions (29). The hallmark of placental pathological findings in patients with APLA syndrome who had a complicated pregnancy is usually thrombosis and evidence of abnormal vascularization of the placenta (29). Therefore, APLA syndrome was regarded as a classical example of a hypercoagulable state that affects pregnancy outcomes.

What is the role of inflammation in APLA syndrome and pregnancy complications?

Recently, a novel approach suggested that the underlying mechanisms in which the APLA exert their effect is through the activation of the complement system (27).

The complement system is the key effector that implicates the activation of inflammation in antiphospholipid syndrome during pregnancy. Although activated complement components are present in normal placentas (30), it appears that in successful pregnancies, uncontrolled complement activation is prevented by three mechanisms: (1) removal of the activated components (31); (2) inhibition of the recruitment of complement receptors on the surface of trophoblastic cells; (3) down-regulation of the complement system (27).

Several mechanisms were proposed to explain the therapeutic effects of heparins (UFH and LMWHs) in the prevention of pregnancy complications in patients with APLA syndrome, based on the pathways activated by the interaction between these antibodies and their targets. Heparins were found to: 1) directly affect the coagulation pathway activation by alloantibodies. During trophoblast differentiation, phosphatidylserine is externalised on the trophoblast outer leaflet where it provides a target for APLA (31–33). Salmon et al. (34) proposed that APLA activate complement within the decidua tissue, thus overwhelming the normally adequate inhibitory mechanisms described above and inducing inflammation and fetal damage. Indeed, by using a murine model of APLA syndrome induced by the passive transfer of human APLA, they demonstrated that complement activation plays an essential and causative role in pregnancy loss and fetal growth restriction (FGR).

Girardi et al. (35, 36) further elucidated this mechanism by demonstrating, in a murine model, that the pathogenic effects of APLA start with their binding to the trophoblast. This interaction can activate the complement system via the classical pathway, with the subsequent involvement of cells and molecules participating in the inflammation process, inducing tissue factor expression, and possibly causing fetal injury (► Figure 3).

The effect of heparins on inflammation and APLA syndrome

Adding heparins to aspirin resulted in a substantial decrease in pregnancy complications in patients with APLA syndrome (3, 37, 38).

Several mechanisms were proposed to explain the therapeutic effects of heparins (UFH and LMWHs) in the prevention of pregnancy complications in patients with APLA syndrome, based on the pathways activated by the interaction between these antibodies and their targets. Heparins were found to: 1) directly affect the coagulation cascade (39); 2) inhibit the binding of β2 glycoprotein I (β2-GPI) to negatively charged phospholipid (40); 3) promote plasmin mediated cleavage of β2-GPI (40); 4) directly inhibit APLA binding to negatively charged phospholipid in APLA immunoassay (41, 42); 5) enhance the clearance of APLA in vivo (43); and 6) inhibit the activation of the complement cascade (44). Moreover, heparins have specific protective effects on the trophoblast in the presence of APLA syndrome. Indeed, they are able to: inhibit binding of APLA to trophoblast cells; promote trophoblast invasiveness; and modulate trophoblast apoptosis (44). However, the role of heparin in the modulation of membrane microvesiculation (45), which is increasingly appreciated to contribute to the hypercoagulability in APLA syndrome, is not yet understood.

Aside from their impact on APLA mediated complement activation, heparins also attenuate the effect of APLA in the cellular

Table 1: Comparison among LMWH preparations. Reproduced with permission from Weitz JI. N Engl J Med 1997; 337: 688–698.

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Method of preparation</th>
<th>Mean molecular weight</th>
<th>Anti-Xa:Anti-IIa ratio*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ardeparin (Normiflo)</td>
<td>Peroxidative depolymerisation</td>
<td>6,000</td>
<td>1.9</td>
</tr>
<tr>
<td>Dalteparin (Fragmin)</td>
<td>Nitrous acid depolymerisation</td>
<td>6,000</td>
<td>2.7</td>
</tr>
<tr>
<td>Enoxaparin (Lovenox)</td>
<td>Benzylisation and alkaline depolymerisation</td>
<td>4,200</td>
<td>3.8</td>
</tr>
<tr>
<td>Nadroparin (Fragiparine)</td>
<td>Nitrous acid depolymerisation</td>
<td>4,500</td>
<td>3.6</td>
</tr>
<tr>
<td>Reviparin (Clivarine)</td>
<td>Nitrous acid depolymerisation, chromatographic purification</td>
<td>4,000</td>
<td>3.5</td>
</tr>
<tr>
<td>Tinzaparin (Innohep)</td>
<td>Heparinase digestion</td>
<td>4,500</td>
<td>1.9</td>
</tr>
</tbody>
</table>

*The ratios were calculated by dividing the anti–factor Xa (anti-Xa) activity by the antithrombin (anti-IIa) activity. The ratios are based on information provided by the manufacturers.
level. Different studies (46–49) have demonstrated an alteration of trophoblast cells by APLA as measured by an increase of apoptosis, a reduction of proliferation and a decrease of the invasive potential. Interestingly, these effects of APLA could be attenuated in vitro by heparin (48). Interaction studies identified a dose-dependent competition between heparin and purified phospholipids in respect to binding with APLA (42). Notably, there is a reduced expression of heparin-binding epidermal growth factor-like growth factor (HB-EGF) in the placental tissue of patients with APLA syndrome (46), which could be improved by heparin inhibiting the binding of APLA.

APLA interactions are primarily ionic in nature, and they bind their targets activating two pathways. The first one is cofactor-dependent, in which these antibodies interact with proteins like β2-GPI (40). A distinguishing feature of IgG APLA are the somatic mutations that lead to the accumulation of positively charged amino acids (arginine, asparagine, and lysine) within the complementary-determining regions of the paratope (50). When realised with proteins, they are a result of the binding of negatively charged sulfonad carboxyl-groups on heparin to positively charged amino acids on the protein (51). The interacting groups within heparin and proteins must be appropriately positioned and oriented to confer the specificity of heparin–protein interactions (51). Further, arginine residues were implicated in the binding of human monoclonal APLA derived from APLA syndrome patients to β2-GPI (52). Indeed, nadroparin (a LMWH) might potentially bind positively charged amino acids within the paratope of APLA (through ionic interactions and/or hydrogen bonds), thus preventing their interaction with β2-GPI (53). This prevents their binding to trophoblast cells and hampers the deleterious effect of these antibodies on trophoblast and pregnancy outcomes. The second mechanism is a cofactor-independent pathway in which APLA interact directly negatively charged phospholipids, like cardiolipins, on the cell membranes (54). It can be hypothesised about an activity of heparin on the interaction between APLA and negatively charged phospholipids resulting in the inactivation of the above mentioned cofactor-independent pathway of cell injury that includes complement and leucocyte activation. This would demonstrate that there can be more than one signalling pathway leading to injury associated to APLA syndrome (54, 55).

Interestingly, as early as 1929, heparin was shown to have “anti-complement effects” (56). Given the importance of complement split products as mediators of APLA-induced fetal injury, Girardi et al. (57) provided convincing evidence that heparin primarily exerts its pregnancy protecting effect via complement inhibition rather than by its anticoagulation capacity. In this study, the influence of different anticoagulants (UFH, LMWH, fondaparinux, and hirudin) on pregnancy outcomes in a model for APLA-associated miscarriage was tested. While all of the anticoagulants tested were able to suppress thrombus formation effectively, only UFH and LMWH protected mice from pregnancy complications and, in parallel, significantly suppressed complement activation. Of interest, fondaparinux, as a synthetic pentasaccharides, uniquely inhibited FXa as well as hirudin blocked thrombin activity but had no effect on the complement activation and were not able to improve pregnancy outcomes in this experimental mouse model. Similar results were presented by Oberkersch et al. (58) that described, in vitro, the anti-inflammatory activity of heparins, exerted by blocking C1q subunit or C1 complex, and by this inhibiting the classical complement pathway. Thus, it is probably the
anti-inflammatory rather than the anticoagulant effect of UFH and LMWH that mediated its potential to prevent pregnancy complications in pregnant patients with APLA syndrome.

Evidence in support of the use of heparin in patients with anti-phospholipid syndrome includes the following: 1) Alalaf et al. (59) compared the efficacy of bemiparin 2,500 U once daily (80 mg/day) vs aspirin (100 mg/day) in women affected by APLA syndrome with at least two consecutive miscarriages. The results suggest a beneficial effect of bemiparin over aspirin (RR for live birth among women treated with bemiparin 1.20, 95% CI 1.00–1.43). The patients’ allocations were not concealed, as the study was quasi-randomised (sequential assignment of treatment), and for unclear reasons more women were allocated to bemiparin compared with aspirin; 2) The evidence for a beneficial effect is strongest for treatment with UFH combined with aspirin when compared with aspirin only in women with APLA syndrome and recurrent pregnancy loss. A meta-analysis showed that in women with two or more pregnancy losses, treatment with UFH combined with aspirin reduced the chance of first trimester miscarriage when compared with aspirin only (n = 109; RR 0.26, 95% CI 0.14–0.48) (60). In two trials in which treatment with LMWH combined with aspirin (n = 96) was compared with aspirin only (n=90), the pooled relative risk (RR) for pregnancy loss was 0.70 and did not reach statistical significance (95% CI 0.34–1.45). When comparing any heparin (UFH or LMWH) combined with aspirin or aspirin alone, the beneficial effect of heparin on reducing the risk of first trimester miscarriage was maintained (RR 0.39, 95% CI 0.24–0.65) with little statistical heterogeneity (I2 10%) (60). Interestingly, in the studies that observed a profound effect of UFH added to aspirin, the chances of a live birth in the aspirin only arms were only 44% and 42% (61, 62), which is much lower than the previously reported chances of a live birth that varied between 68% and 80% (63–65); 3) The comparison of the effect of LMWH vs UFH (66–68) or between different concentrations of the same LMWH drug (37) did not yield significant differences.

UFH with low-dose aspirin was regarded as the treatment of choice for the prevention of recurrent pregnancy loss in women with APLA syndrome. In the last decade, with the introduction of LMWH, there is a tendency to replace UFH with LMWH. Recent meta-analyses suggest that this tendency deserves a second thought. The first systematic review and meta-analysis showed that in women with APLA syndrome and two or more pregnancy losses, treatment with UFH plus aspirin reduced the chance of first trimester miscarriage when compared with aspirin only (OR 0.26, 95% CI 0.14–0.48, number needed to treat of 4) (60). The combination of LMWH plus aspirin did not have the same beneficial effect as aspirin alone. In addition, there was no difference between UFH plus aspirin vs LMWH plus aspirin, even though the studies included in the meta-analysis were based on a small number of patients. A systematic review and meta-analysis (69) from 2014 evaluated all the randomised clinical trials (RCTs) examining the use of pharmacotherapies in pregnant women with APLA syndrome. The authors found an increased live birth rate following the use of a combination of aspirin and UFH compared to aspirin alone, whereas the association between aspirin and LMWH resulted in a rate similar to aspirin alone. ▶ Table 2 summarises the RCTs relating to the treatment with UFH and low-dose aspirin as well as LMWH and aspirin for the prevention of recurrent pregnancy loss in women with APLA syndrome. These RCTs are based on a relatively low number of patients, and it might be that they are underpowered to detect a true difference in the effectiveness of these medications on recurrent pregnancy loss.

Collectively, the evidence presented herein suggests that heparins are a useful treatment for the prevention of pregnancy complications in women with APLA syndrome due to their anti-inflammatory activity against complement activation, as well as through their cellular effect on trophoblast. Moreover, the benefit of the use of UFH over LMWH should be the subject of future debate because it is not clear if there is a real difference in their effect in the prevention of pregnancy complications in patients with APLA syndrome. Further studies, including those with a larger number of patients, are needed to clarify this point.

**Heparin as a treatment for recurrent pregnancy loss**

A critical point is represented by the definition of “recurrent” pregnancy loss. The Royal College of Obstetricians and Gynaecologists (RCOG) considers three or more first trimester miscarriages as recurrent pregnancy loss, whereas the American Society for Reproductive Medicine (ASRM) states that two or more pregnancy losses are enough to diagnose this condition.

Recurrent pregnancy loss is caused by many underlying mechanisms, including uterine malformations (70), chromosomal defects (71), endocrinopathies (72) (thyroid disease and diabetes), and autoimmune diseases (73). In addition, during the turn of the 21st century, thrombophilic mutations (74) have been proposed as an additional underlying mechanism. These mechanisms lead to problems in embryonic formation, migration, implantation and placentation.

In light of the possible effects of heparins and heparin binding molecules on the blastocyst implantation and placentation, this family of drugs may play a role in the treatment of patients with recurrent pregnancy loss. Indeed, a beneficial effect of antithrombotic agents, heparin in particular, in women with recurrent pregnancy loss was hypothesised as early as 1980 (75). In the past three decades, this area became a field of extensive study, with the goal of increasing the rate of live births in these patients.

**What is the effect of heparins on pregnancy success in women with recurrent pregnancy loss and thrombophilia?**

The studies performed to address this question yielded conflicting results; moreover, the marked heterogeneity among the different studies affects our ability to draw a convincing conclusion from them. Evidence in support of this view is brought herein: 1) In a prospective cohort study (38, 50) women with recurrent pregnancy loss and thrombophilia were treated with enoxaparin (40 mg
daily for single and 80 mg daily for combined thrombophilic effects). Live births occurred in 75% of gestations, with an increase from 20% in previous pregnancies. The design of such a study has been criticised based on regression to the mean by using the previous bad pregnancy outcome as a control (76); 2) In the LIVE-ENOX study (77), 180 women with thrombophilia and recurrent pregnancy loss were randomised to either enoxaparin 40 or 80 mg once daily. In addition to inherited thrombophilia, women with APLA, MTHFR 677TT genotype and hyperhomocysteinaemia were eligible. The live birth rates in both groups were similar (84.3% and 78.3%, respectively), but as a control group was lack-

age of six weeks. Of these women, 299 became pregnant. The chance of live birth did not differ between the treatment groups. The RR of live birth for women who became pregnant was 1.03 (95% CI 0.85–1.25) for nadroparin combined with aspirin and 0.92 (95% CI 0.75–1.13) for aspirin only compared with placebo. The study was not designed to evaluate the beneficial effect of heparin on thrombophilic patients but the subgroup analysis of patients with or without inherited thrombophilia was included among the results, showing no significant difference in the primary outcome among the groups; 2) In addition, two studies randomised women to different antithrombotic treatment regimens but lacked a control arm with a placebo or no treatment. The results showed no difference in the live birth rates between these treatments (79, 82); 3) According to a Cochrane review (83), based upon an extensive literature search from 1966 to 2004 of women with a history of two or more spontaneous losses or a fetal demise without apparent cause other than inherited thrombophilia, only two trials were available for review. The first study was conducted by Gris et al. (84) and compared the administration of low-dose aspirin at a dose of 100 mg daily (n=80) with 40 mg daily

### Table 2: RCTs evaluating live birth rate in patients with recurrent pregnancy loss associated to antiphospholipid syndrome.

<table>
<thead>
<tr>
<th>Type of comparison</th>
<th>Study design</th>
<th>Number of participants</th>
<th>Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>LMWH plus LDA vs UFH plus LDA</td>
<td>Randomised pilot trial in pregnant patients with APLA syndrome (100)</td>
<td>28</td>
<td>Dalteparin may be an effective alternative to UFH for treatment of APLA syndrome in pregnancy.</td>
</tr>
<tr>
<td></td>
<td>Prospective, multicentre, controlled pilot study in patients with APLA syndrome and RPL (101)</td>
<td>50</td>
<td>No statistical difference between the two groups</td>
</tr>
<tr>
<td></td>
<td>Randomised prospective study in patients with APLA syndrome and RPL (102)</td>
<td>60</td>
<td>LMWH plus LDA was successfully used as an alternative to UFH plus LDA in the management of recurrent abortion secondary to APLA syndrome.</td>
</tr>
<tr>
<td>LMWH plus LDA vs LDA alone</td>
<td>RCT in pregnant patients with APLA syndrome and RPL (103, 104)</td>
<td>98</td>
<td>The addition of low molecular weight heparin does not significantly improve pregnancy outcome respect to the use of aspirin alone</td>
</tr>
<tr>
<td></td>
<td>RCT in pregnant patients with APLA syndrome, ANA, or an inherited thrombophilia and RPL (104)</td>
<td>88 (42 with APLA syndrome)</td>
<td>LMWH/LDA did not confer incremental benefit compared to LDA alone</td>
</tr>
<tr>
<td>UFH plus LDA vs LDA alone</td>
<td>Prospective, single-centre, trial with alternative assignment to treatment of pregnant patients with APLA syndrome and RPL (105)</td>
<td>50</td>
<td>Heparin plus low-dose aspirin provides a significantly better pregnancy outcome than low-dose aspirin alone does for antiphospholipid antibody-associated recurrent pregnancy loss.</td>
</tr>
<tr>
<td></td>
<td>RCT in pregnant women with recurrent miscarriage associated with APLA syndrome (106)</td>
<td>90</td>
<td>Treatment with aspirin and heparin leads to a significantly higher rate of live births in women with a history of recurrent miscarriage</td>
</tr>
<tr>
<td></td>
<td>RCT in pregnant patients with RPL and APLA syndrome (107)</td>
<td>72</td>
<td>Treatment with a combination of aspirin and heparin showed better outcome than treatment with aspirin alone</td>
</tr>
</tbody>
</table>

LMWH, low-molecular-weight heparin; UFH, unfractionated heparin; LDA, low-dose aspirin; RCT, randomised controlled trial; RPL, recurrent pregnancy loss; APLA, antiphospholipid antibodies.
(n=80) enoxaparin from the 8th week of gestation in a cohort of patients with a prior pregnancy loss after 10 weeks and the presence of heterozygous factor V Leiden (FVL), prothrombin gene mutation (PGM), or protein S (PS) deficiency. The authors found that enoxaparin treatment was better than aspirin alone (OR 15.5; 95 % CI: 7–34; P < .0001). The birthweights were higher, and there were less small for gestational age (SGA) infants in the enoxaparin group. Nevertheless, this study was criticised regarding the concealment of allocation, lack of generalisability due to very strict inclusion criteria, and the fact that women who experienced an early miscarriage after randomisation were not taken into account. The other study was the trial reported by Tulppala et al. (64) that involved 82 patients and compared 50 mg of aspirin with a placebo starting at the time of a positive urine pregnancy test in women with three or more unexplained consecutive pregnancy losses. No differences were noted in the aspirin compared with the placebo group (RR 1.00, 95 % CI 0.78–1.29).

Little evidence is available for the effect of antithrombotic agents in women with a single pregnancy loss and inherited thrombophilia. The results from several small retrospective and prospective cohort studies in women with inherited thrombophilia, with or without previous pregnancy complications, suggest a beneficial effect of antithrombotic therapy to reduce pregnancy complications (2, 85). These studies are heterogeneous with regard to study design and study population.

In light of the evidence presented above, UFH and LMWH may have a role in improving pregnancy outcomes among a subset of patients with recurrent pregnancy loss regardless to the presence of thrombophilia, but still further studies are needed because the data from current studies remain conflicting.

### The use of heparin for the prevention of pregnancy complications

Over the last 10 years, obstetricians started to adopt antithrombotic prophylaxis in the majority of women considered at risk of adverse events due to past late pregnancy complications, or women with a history of recurrent abortions (86).

Although the pathogenesis of the great obstetrical syndromes remains unclear, these complications have been associated, in different ways, with abnormal placental perfusion and alterations in the haemostatic process. In addition, placental multiple infarcts, spiral artery thrombosis, perivillous fibrin deposition and thrombosis of the fetal stem vessels (87–89) as well as increased thrombin generation in the maternal circulation (1, 24), are frequently observed.

Nevertheless, the clinical trials implementing antithrombotic prophylaxis with heparin or its analogues for the prevention of these syndromes, yielded conflicting results (90, 91). In addition to the small number of randomised controlled trials (RCTs), the lack of a placebo arm is one of the greatest limitations of these studies. Indeed, the option of no treatment or placebo is very difficult to be accepted by women with prior pregnancy complications. This may result from information obtained through the web sources on the use of LMWH to prevent recurrent pregnancy complications.

### Evidence about the use of heparin in the prevention of obstetric complications

A pilot randomised trial (90) compared dalteparin 5,000 units daily vs no prophylaxis in 110 women without identifiable thrombophilia who had prior placental-mediated complications. Non-thrombophilic women were included if they had previously experienced the following: 1) severe preeclampsia necessitating delivery prior to 34 6/7 weeks; 2) unexplained birthweight <5th percentile; or 3) placental abruption necessitating delivery prior to 34 6/7 weeks or resulting in fetal death after 19 6/7 weeks. Dalteparin was associated with a lower rate of the composite primary outcome that included severe preeclampsia, birth weight less than the 5th percentile, and major placental abruption (leading to birth prior to 34 weeks or fetal death after 20 weeks). This composite outcome occurred in 5.5 % of those in the dalteparin arm compared to 23.6 % in the no prophylaxis arm (OR 0.15, 95 % CI 0.03–0.70, number needed to treat 5.5, p = 0.016). However, these results need to be interpreted with caution as the trial did not reach the intended sample size of 276 women nor did the interim analysis of the primary outcome reach the pre-planned level of statistical significance (p < 0.005). Furthermore, stopping trials early due to efficacy is known to exaggerate treatment effects. Kupferminc et al. (89) studied 72 women with a history of pregnancy complications (severe preeclampsia, FGR <5th percentile, severe placental abruption and/or stillbirth >20 weeks) or placental vasculopathy, who had a negative workup for thrombophilia. The study group (n=32) was treated with LMWH, and the control group (n=40) received no treatment. The incidences of severe preeclampsia, FGR, placental abruption and stillbirth in the previous pregnancies were similar in both groups. The incidences of severe preeclampsia and placental abruption in the study group in the index pregnancy were significantly lower in the treatment than in the control group (3.13 vs 20 %, p = 0.03; and 0 vs 15 %, p = 0.03, respectively). The respective incidence of FGR was 6.25 % vs 22.5 %, and the rate of overall adverse outcome was 9.4 % vs 60 % (p = 0.001) in the treatment vs control group. The authors suggested that treatment with LMWH may reduce the rate of recurrent severe pregnancy complications in women without thrombophilia.

Mello et al. (92) randomised 80 women with angiotensin converting enzyme deletion (ACE DD) polymorphisms and a history of preeclampsia without known thrombophilia to treatment with dalteparin 5,000 units vs no treatment. Women in the dalteparin group had a significant reduction in the risk of recurrent preeclampsia, early onset preeclampsia (<34 weeks of gestation) and FGR in comparison to the non-treatment group [recurrent preeclampsia 7.3 % (3/41) vs 28.2 % (11/39); early onset preeclampsia 2.4 % (1/41) vs 20.5 % (8/39); FGR 9.8 % (4/41) vs 43.6 % (17/39), respectively].

The use of UFH/LMWH for the prevention of pregnancy complications in women with inherited thrombophilia is controversial.
Several studies evaluated the benefit of the treatment with LMWH or aspirin in the prevention of pregnancy complications in patients with inherited thrombophilia and a history of pregnancy complications (preeclampsia, IUGR, recurrent pregnancy loss, fetal demise, and placental abruption). Most of these studies use enoxaparin and dalteparin in the heparin arm; however, one of the main pitfalls of many of them is the lack of a placebo arm (2, 3, 38, 93).

In particular, dalteparin, in combination with aspirin, administered to women with inherited thrombophilia and previous pregnancy complications significantly decreased the risk of preeclampsia by 20% (OR 0.80; 95% CI 0.70–0.91) and FGR by 30% (OR 0.70; 95% CI 0.60–0.82) (94). Stronger evidence has been reported in a RCT performed in 2011 by De Vries et al. (95) in the Netherlands. They randomized 139 women with previous indicated preterm delivery <34 weeks due to early onset hypertensive disorders of pregnancy or small for gestational age and inheritable thrombophilia in two arms receiving either dalteparin with low-dose aspirin or aspirin alone. All participants were less than 12 weeks of gestation at enrollment and were negative for APLA. The investigators found a reduction in the risk of recurrent hypertensive disease <34 weeks of 8.7% (CI 1.9–15.5) with a number needed to treat of 12. However, the overall rate of recurrent hypertensive disease did not differ among the groups (96). The importance of this study is that it showed that through targeted treatment, we may be able to reduce the severity of the disease and postpone its clinical presentation to late stages of pregnancy.

In contrast, in a randomised open-label controlled trial [Thrombophilia in Pregnancy Prophylaxis Study (TIPPS)] (97), patients at high risk for pregnancy complications with confirmed thrombophilia were randomised to receive either dalteparin (5,000 units/day until 20 weeks and 5,000 units every 12 h until 37 weeks or onset of labour) or no treatment. No effect of dalteparin in reducing coagulation activation was described. The effect of dalteparin on thrombin-antithrombin III (TAT-III) complexes concentrations was defined as the primary outcome. Dalteparin prophylaxis resulted in a significant increase in anti-Xa activity through pregnancy (p<0.0001) compared to the controls but had no significant effect on TAT-III, prothrombin fragments 1+2 and D-dimer concentrations throughout pregnancy in these women.

The limit of this study is due to its inclusion criteria. In addition to being thrombophilic patients, participants had to have at least one of the following eligibility criteria: previous preeclampsia, previous unexplained IUGR, previous recurrent pregnancy losses under 16 weeks of gestational age or a fetal loss at >16 weeks of gestational age, previous major placental abruption, previous venous thromboembolism, or a first degree relative with symptomatic thrombophilia. Because the inclusion criteria were not stratified by the early or late onset of diseases, this study did not allow an evaluation of the efficacy of heparins in terms of the severity of the diseases or their onsets during gestation.

In their recently published Cochrane review, Dodd et al. (98) suggested that women considered at high risk of adverse pregnancy complications secondary to placental insufficiency can benefit from treatment with heparin. This is based on a statistically significant reduction in risk of perinatal mortality (six studies; 653 women; RR 0.40; 95% CI 0.20–0.78), preterm birth <34 (three studies; 494 women; RR 0.46; 95% CI 0.29–0.73) and <37 weeks’ gestation (five studies; 621 women; RR 0.72; 95% CI 0.58–0.90), and infant birthweight below the 10th centile for gestational age (seven studies; 710 infants; RR 0.41; 95% CI 0.27–0.61), when compared with no treatment.

Collectively, these data suggest that in a well-selected population with a history of previous early onset preeclampsia or IUGR, with or without thrombophilia, a prophylactic treatment with UFH/LMWH may reduce the recurrence of these complications, or postpone their development during gestation. However, the greatest question to be answered is “how can we identify those who will benefit for such a treatment?”

Summarisation

The data presented herein suggest that the administration of UFH or LMWH can be beneficial in the prevention of pregnancy complications in a selected population. However, the definition of which patients will benefit from such treatment for a secondary prevention of these obstetrical syndromes is not well defined. For example, the use of UFH or LMWH as a primary prevention of preeclampsia or IUGR in a population at risk, such as women with inherited thrombophilia, was not proven to be beneficial. Indeed, not all patients with thrombophilia will develop pregnancy complications. Therefore, there is a need for a functional assay in order to assess in real time those patients with increased thrombin generation (with and without thrombophilia) that are at increased risk for these complications, and identify candidates who will benefit from the treatment with UFH or LMWH. Preliminary evidence suggests that the use of thrombin generation assays (1) or platelet function tests may be useful for this purpose (99).

Conflicts of interest

None declared.

References


Mastrolia et al. Heparins and pregnancy complications