TITLE PAGE

Low-molecular-weight heparin for prevention of preeclampsia: a systematic review and meta-analysis.

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CONFLICTS OF INTEREST

Dr. Llurba reports receiving fees for lectures from Sanofi and serving on advisory boards from Roche Diagnostics. The remaining authors report no conflicts of interest.

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CONDENSATION

Based on the evidence currently available, if LMWH is considered for prevention of preeclampsia in high-risk women, combination with LDA is recommended.

SHORT TITLE

Low-molecular-weight heparin for prevention of preeclampsia.

AJOG AT A GLANCE

A. Why was this study conducted?

- The role of heparin for prevention of preeclampsia (PE) is tested in small trials with many limitations and conflicting results.

B. What are the key findings?

- Combination of low-molecular-weight heparin (LMWH) and low-dose aspirin (LDA) performs better than LMWH alone for prevention of PE.

C. What does this study add to what is already known?

- These effects seem to be maintained regardless of the presence of thrombophilia.
- No increased risk of hemorrhage or placental abruption was found with LMWH alone or in combination with LDA.
- Enoxaparin appears to be more effective, over Dalteparin.

ABSTRACT

Background

Previous meta-analyses suggest that intervention with low-molecular-weight heparin (LMWH) is not effective for prevention of preeclampsia (PE) in high-risk patients. However, LMWH's effect in preventing such conditions is likely to be higher than that previously observed, if specific groups of high-risk women are selected or if it is combined with LDA.

Objective

To assess the effectiveness of LMWH in the prevention of PE and other placenta-related complications.

Data sources

Systematic search performed to identify relevant studies, using the databases PubMed and Cochrane Central Registry of Controlled Trials, without publication time restrictions.

Study eligibility criteria

Randomized controlled trials (RCTs) that compared treatment with LMWH or unfractionated heparin (with or without low-dose aspirin, LDA), to those without intervention or LDA alone, in women at high-risk of PE.

Study appraisal and synthesis methods

The systematic review was conducted according to the Cochrane Handbook guidelines. The primary outcome was development of PE. Secondary outcomes included SGA, perinatal death, miscarriage and placental abruption (PA). Pooled odds ratio (OR) with 95% confidence intervals (95% CI) were calculated using a random-effects model. Quality of evidence was assessed using the GRADE methodology.

Results

A total of 15 studies (2848 participants) were included. In high-risk women, treatment with LMWH was associated with reduction in development of PE (OR 0.64; 95%CI 0.45-0.92); SGA (OR 0.62; 95%CI 0.44-0.88), miscarriage (OR 0.57; 95%CI 0.33-0.97); preterm delivery (OR 0.72; 95%CI 0.51-1.00), and perinatal death (OR 0.26; 95%CI 0.07-0.98). However, this reduction only remained significant if LMWH was combined with LDA (7 studies, 1092 participants) for PE (OR 0.50; 95%CI 0.35-0.73) and SGA (OR 0.52; 95%CI 0.36-0.74), with no effect found with LMWH alone. Sub-analysis of LHWH type showed that enoxaparin performed better than dalteparin for prevention of PE. Overall, adverse events were neither serious nor significantly different. Quality of evidence ranged from very low to moderate, mostly due to the lack of blinding, imprecision and inconsistency.

Conclusions

LMWH decreases the risk of PE and other placental mediated complications in high-risk women; however, this decrease remains statistically significant only when LMWH is combined with LDA. Based on the evidence available so far, if LMWH therapy is considered for high-risk women, combination with LDA is recommended.

KEYWORDS

Aspirin, Fetal growth restriction, Low-molecular-weight heparin, Placental insufficiency, Preeclampsia, Prevention.

MAIN TEXT

INTRODUCTION

Prevention of placenta-mediated complications is likely the best approach to reduce maternal and perinatal mortality and morbidity associated with Preeclampsia (PE) and fetal growth restriction (FGR).[1-4] Previous attempts at prevention of PE using prophylactic interventions from mid-gestation have been largely unsuccessful.[5-7] Low-dose aspirin (LDA) is effective in preventing PE and FGR, but only when treatment is initiated before 16 weeks of gestation.[8] Therefore, its use should be considered standard practice for women at high-risk of developing PE.[9]

In the first trimester, PE and FGR can be predicted with 60–80% sensitivity based on maternal history, uterine artery mean pulsatility index (PI) Doppler assessment, and biochemical markers.[10, 11] A recent study demonstrated that intervention with LDA following first trimester screening of women at risk of developing PE, resulted in >50% reduction in occurrence of preterm PE (odds ratio [OR] in the aspirin group, 0.38; *p*=0.004).[12] Therefore, strategies for implementing such screening in routine clinical practice are being considered.[13] However, women selected based on their obstetric history or medical risk factors account for only a small proportion of those women selected from first trimester screening, and they constitute a particularly high-risk group for development of the earliest and severest form of PE.

Since thrombosis in the uteroplacental circulation is frequently observed in severe and early forms of placenta-mediated complications, anticoagulation could potentially improve placental perfusion. Low-molecular-weight heparin (LMWH) has known antithrombotic effects and can promote differentiation and invasion of trophoblasts *in vivo*.[14] LMWH can also prevent

monocyte adhesion, inhibit tumor necrosis factor,[15] and decrease vascular resistance and endothelial function.[16] Therefore, it is speculated that LMWH might improve placental development and inhibit reactive pathways involved in PE and FGR. However, data on LMWH in women without thrombophilia are very limited.[17-19] Although a more recent study showed promising results,[20] an individual patient meta-analysis failed to demonstrate a benefit for LMWH in patients at risk of placental-mediated complications.[21] Moreover, two recent randomized trials in high-risk patients showed that addition of enoxaparin to standard care did not reduce the risk of recurrence of PE and FGR.[22] However, the effect of heparin in preventing such conditions is likely to be higher than previously observed, if specific groups of high-risk women are selected or if heparin is combined with LDA.[23, 24]

OBJECTIVES

The objective of this meta-analysis was to assess the effectiveness of LMWH in the prevention of PE and other placenta-related complications. As secondary objectives, analyses of the combined treatment of LMWH and LDA, presence of thrombophilia, moment of initiation of treatment, type of heparin administered, and study-center effects were performed. Risk of hemorrhagic complications or secondary effects of LMWH were also evaluated. Finally, a sub-analysis was performed to ascertain differences among type of heparin administrated.

METHODS

This systematic review was conducted in accordance to guidelines of the Cochrane Handbook for Systematic Reviews of Interventions[25] and reported in agreement with the

Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.[26] The study protocol was registered with PROSPERO, number CRD42020191148. We included in this meta-analysis randomized controlled trials (RCTs) that compared treatment with LMWH or unfractionated heparin (with or without LDA) to those who received no treatment or LDA alone, in women at high-risk of PE.

Eligibility criteria, information sources, search strategy

An electronic search was made of PubMed and Cochrane Central Registry of Controlled Trials to identify studies published from 1945 to June 2020. We reviewed abstracts of congresses and scientific meetings, reference lists of retrieved articles, published study protocols, previously published systematic reviews, and review articles for any additional relevant studies. No language restriction was imposed. A combination of the following keywords and MeSH terms were included: “heparin”, “Low-Molecular-Weight”, “LMWH”, “aspirin”, “preeclampsia”.

Study selection

We included RCTs on women who had any known risk factors for development of PE, such as adverse obstetric history (previous PE, FGR, placental abruption or stillbirth), and medical history including thrombophilia, autoimmune diseases and chronic hypertension. Trials were excluded if they: (1) were not randomized; (2) assessed the effect of heparin on diagnosed PE; or (3) did not report PE as an outcome. Studies were also excluded if additional information on methodological issues or complete results could not be obtained. If co-interventions were

present, we considered eligible for inclusion provided they were present equally for each trial arm.

The primary outcome was development of PE (mild or severe, term or preterm), with secondary outcomes including FGR or small-for-gestational-age (SGA), stillbirth, perinatal death, miscarriage, PA, preterm delivery, hemolysis, elevated liver enzyme and low platelet (HELLP) syndrome, eclampsia and maternal death. We also analyzed the presence of adverse events such as bleeding or hemorrhage, treatment-related allergies, and thrombocytopenia. Criteria for the definition of PE were those of the International Society for the Study of Hypertension in Pregnancy[27] or those of the American College of Obstetricians.[28] Considering variations in the definition of PE around the world, similar definitions were accepted.[29] SGA was defined as neonatal birth weight below the 10th, 5th or 3rd percentile (the first available in this order), or fetal estimated weight below 10th percentile.[30]

Additionally, we decided to perform sub-analyses of the studies based on 5 important points for clinical practice: combination of LMWH with LDA, moment of initiating treatment, presence of thrombophilia, type of heparin administered and study-center effects

Data extraction

Record screening by abstract revision was performed independently by two of the authors (M.CL. and J.U.). Final selection, with full-article review and data extraction to a specifically developed form, was performed independently by three reviewers (M.CL., J.U. and E.L.) and any discrepancies were resolved by discussion. Information was extracted on study characteristics (randomization procedure, mono- or multi-centric), participants (inclusion and

exclusion criteria, number of women per group, presence and type of risk factors, maternal age, gestational age at inclusion), details of interventions (heparin type, daily dosage, presence of cointervention with aspirin, use of placebo) and outcomes (number of outcome events, adverse effects reported). JC.V. assessed risk of bias of the final included studies and performed data analysis.

Assessment of the risk of bias

The risk of bias of the RCTs was evaluated using the Risk of bias tool of the Cochrane Collaboration.[31] Specifically, domains were evaluated for selection bias, detection bias and attrition bias. In presence of bias in any of these aspects, the trial was deemed as high-risk of bias. In addition, we also assessed the performance bias and the report bias of the outcomes of interest. The information obtained from the evaluation of the risk of bias was incorporated to classify quality of the evidence for the primary outcomes as high, moderate, low or very low according to the methodological guidelines of the GRADE system.[32, 33]

Data synthesis

From the data obtained from the studies, Odds Ratios (ORs) with the 95% confidence intervals (95% CI) were calculated. Meta-analyses were performed when appropriate using the Mantel-Haenszel random effects model, because we anticipated the presence of significant heterogeneity, measured with the I^2 statistic (heterogeneity was considered as significant when I^2>50%).[31] We calculated the estimates of effect and obtained forest plots using Review Manager (Version 5.3; Nordic Cochrane Centre, Copenhagen, Denmark) program.

The flow diagram used to identify and select the trials is described according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement.[26]

RESULTS

Study selection and characteristics

Figure 1 summarizes the process of identification and selection of studies. Fifteen studies which included 2848 women met the inclusion criteria.[22, 24, 34-46] The main characteristics of the studies included in this systematic review are shown in Table 1.

Risk of bias of included studies

Overall, methodological quality ranged from moderate to very low due to concerns about risk of bias (blinding was not possible in most trials because of the intervention, i.e. subcutaneous injection of LMWH), the small number of events for many outcomes, lack of details about important methodological issues in some studies, substantial heterogeneity detected in the analyses, and wide confidence intervals indicating imprecision in the results. All trials except two,[34, 39] were considered as high-risk of bias due to lack of blinding, but even for these with unclear risk of bias, it was unlikely to have occurred. Methodological quality assessment for each individual study, risk of bias graph and summary can be found in the Supplementary Material.

Synthesis of results

Primary outcome: Preeclampsia

The meta-analysis with data from the 15 trials included (2848 women) showed that patients treated with LMWH presented significantly less PE than those not treated with LMWH (OR 0.64; 95%CI 0.45-0,92; *p*=0.020; I^2=31%). This association was particularly significant for the group of patients where LMWH was combined with LDA (7 RCT,[35-39, 43, 45] 1092 participants, OR 0.50; 95%CI 0.35-0.73; *p*=0.0003; I^2=0%). Figure 2 shows forest tree plots for both analyses. Quality of evidence according to GRADE criteria was moderate, due to the lack of blinding, the small number of events and lack of details about important methodological issues. Complete GRADE assessment can be found in the Supplementary Material.

Subgroup analyses performed for early-onset PE (<34 weeks) showed a statistically non-significant reduction with administration of LMWH, with substantial heterogeneity (Figure 3; 7 RCTs,[22, 35, 36, 38, 42, 45, 46] 1109 participants, OR 0.43 95%CI 0.17-1.09, p=0,070, I2=54%). For timing of treatment analyses, 2 studies where treatment began after 16 weeks of gestation were excluded;40, 46 the impact of LMWH on PE was stronger than in the global meta-analysis (Figure 4; 13 RCTs, 2527 participants, OR 0.57, 95%CI 0.42-0.78, p=0.0005, I2=10%). No differences were observed when considering presence of thrombophilia; it did not modify the effect of LMWH on occurrence of PE (Figure 5). In subgroup analysis by type of LMWH, we found that enoxaparin was associated with a significant reduction in PE, while in patients treated with dalteparin, such reduction did not reach statistical significance (Figure 6. Enoxaparin: 7 RCTs,[22, 24, 34, 36-39] 1585 participants; OR 0.56; 95%CI 0.37-0.85; p=0.006; I2=25%; Dalteparin: 6 RCTs,35, 42-46 1103 participants; OR 0.60; 95%CI 0.33-1.10; p=0.100; I2=17%). Finally, subgroup analysis by type of study showed that studies conducted in one center were more likely to be associated with a

statistically significant decrease in the development of pre-eclampsia, compared to studies conducted in more than one center/country (Unicenter: 6 RCTs, 1010 participants; OR 0,41; 95% CI 0,26 to 0,64; p < 0,0001; I2 = 47% versus Multicenter: 9 RCTs, 1838 participants; OR 0,84; 95% CI 0,59 to 1,18; p = 0,32; I2 = 0%).

Secondary outcomes

Figures for secondary outcomes may be found in the Supplementary Material.

Small-for-gestational-age

Data from the 15 trials included showed that patients treated with LMWH presented significantly less SGA than those not treated with LMWH (15 RCTs, 2848 participants; OR 0.62; 95%CI 0.44-0.88; *p*=0.007; I^2=52%); the association was also significant for the group of patients where LMWH was combined with LDA (OR 0.52; 95%CI 0.36 0.74; p=0.0003; I^2=0%) (Figure 7). Subgroup analysis for timing of treatment did not lead to a substantial change in its effect, or in the heterogeneity (Figure S1; 13 RCTs, 2527 participants; OR 0.64; 95% CI 0.44-0.93; *p*=0.020; I^2=55%). Presence of thrombophilia did not modify the effect of LMWH on the occurrence of SGA (Figure S2). Subgroup analysis by type of heparin showed dalteparin associated with a significant reduction in development of SGA, while in patients treated with enoxaparin, such reduction did not reach statistical significance (Figure S3. Enoxaparin: 7 RCTs, 1585 participants; OR 0.78; 95%CI 0.50-1.22; *p*=0.270; I^2=58%; Dalteparin: 6 RCTs, 1103 participants; OR 0.48; 95%CI 0.28-0,82; *p*=0.007; I^2=29%).

Stillbirth and perinatal death

No statistically significant differences were found in the occurrence of stillbirth between LMWH-treated and non-treated patients (11 RCTs, 2364 participants; OR 0.71; 95%CI 0.39-1.27; p=0.25; I^2=0%), regardless of whether LMWH was combined with LDA (Figure S4). Significantly fewer perinatal deaths occurred among the offspring of LMWH-treated patients, compared to non-treated patients (Figure S5; 4 RCTs,[22, 38, 40, 45] 473 participants; OR 0.26; 95%CI 0.07-0.98; p=0.05; I^2=15%).

Miscarriage

8 RCTs[35-39, 43, 45, 46] (1381 women) reported the outcome of miscarriage. Patients treated with LMWH had significantly fewer miscarriages than non-treated patients (OR 0.57; 95%CI 0.33-0.97; p=0.040; I^2=0%). Only one trial[46] did not administer LDA in the non-LMWH group (Figure S6). Two subgroups analyses were performed for this outcome; no differences were observed regarding the presence of thrombophilia. In subgroup analysis by type of LMWH, enoxaparin was also associated with a significant reduction of miscarriage, while dalteparin did not reach statistical significance (Figure S7; Enoxaparin: 4 RCTs, 807 participants; OR 0.40; 95%CI 0.20-0.84; p=0.020; I^2=0%; Dalteparin: 4 RCTs, 574 participants; OR 0.84 95%CI 0.39-1.85; p=0.67; I^2=0%).

Placental abruption

No statistically significant difference in the occurrence of PA was found between LMWH-treated and non-treated patients, regardless of combination with LDA (Figure S8. 14 RCTs, 2768 participants; OR 1.01; 95%CI 0.66-1.56; p=0.96; I^2=0%).

Preterm delivery

Patients treated with LMWH had fewer preterm deliveries than non-treated patients, but this was not statistically significant (14 RCTs, 2768 participants; OR 0.72; 95%CI 0.51-1.00; *p*=0.05; I^2=62%). The effect in the subgroup of trials where LMWH was combined with LDA did not reach statistical significance (Figure S9). For timing analyses, LMWH initiated before 16 weeks led to an increase in the treatment effect, reaching statistical significance (Figure S10. 12 RCTs, 2447 participants; OR 0.66; 95%CI 0.46-0,95; *p*=0.02; I^2=64%). No differences were observed when considering the presence of thrombophilia; it did not modify the effect of LMWH on the occurrence of preterm birth. Finally, in the subgroup analysis by type of LMWH, we found that enoxaparin was associated with a significant reduction of preterm deliveries, while in the subgroup of patients treated with dalteparin, such reduction did not reach statistical significance (Figure S11. Enoxaparin: 7 RCTs, 1585 participants; OR 0.59; 95%CI 0.35-0.98; *p*=0.04; I^2=76%; Dalteparin: 5 RCTs, 1023 participants; OR 0.95; 95%CI 0.60-1.51; *p*=0.84; I^2=36%).

Other outcomes: HELLP syndrome, eclampsia and maternal death

No statistically significant differences were found in terms of the occurrence of HELLP syndrome between LMWH-treated and non-treated patients, regardless of whether or not heparin was combined with LDA (Figure S12. 8 RCTs, 1242 participants; OR 0.78; 95%CI 0.33-1.88; *p*=0.59; I^2=0%). 7 RCTs reported the occurrence of eclampsia, although 4 of them reported no events in any of the groups.[22, 24, 35, 38, 39, 41, 45] One study[39] reported 3 episodes of eclampsia, 1 in the LMWH group and 2 in the non-treated group. 3 studies[22, 38, 44] reported maternal mortality as an outcome, with no events in either group.

Adverse events

No statistically significant difference in bleeding was found between LMWH-treated and non-treated patients, regardless of whether or not LMWH was combined with LDA (Figure 8. 12 RCTs, 2528 participants; OR 1.10; 95%CI 0.78-1.56; *p*=0.57; I^2=0%). Significantly more episodes of allergy occurred in patients treated with LMWH, when compared non-treated patients (5 RCTs, 1078 participants; OR 10,83; 95% CI 2,17 to 54,10; p = 0,004; I^2 = 54%). However, the number of events was small, and the 95% confidence intervals were too wide to be certain about these results (Figure 9). With the exception of bleeding and allergies, other adverse effects reported were very rare. Thrombocytopenia was reported in 3 studies,[24, 34, 38] with a range between 0 and 6 events; thrombosis was reported in 3 studies,[24, 34, 35] with a range between 0 and 4 events, and finally, another study reported similar numbers of transfusion events in both groups[38].

COMMENT

Main findings

The results of this systematic review and meta-analysis suggest that in high-risk women, LMWH prophylaxis may decrease the risk of PE, delivery of a SGA neonate, miscarriage and perinatal death; however, we found for the first time this depends greatly on its combination with LDA. The observed benefit also seems to be conditioned on beginning treatment before 16 weeks' gestation, specially to prevent PE and SGA. These effects appear to be maintained, regardless of presence of thrombophilia. Women treated with enoxaparin seem to have better outcomes, with the exception of SGA, when compared to dalteparin. In addition, no major side

effects such as PA or hemorrhage were observed in women treated with LMWH (alone or in combination with LDA).

Comparison with existing literature

In a meta-analysis of 7 randomized trials that included 987 pregnant women with previous placental-related complications, prophylactic LMWH significantly reduced the incidence for a primary composite outcome (PE, SGA <10th centile, PA, or pregnancy loss >20 weeks; RR 0.57, 95% CI: 0.36-0.91), however this meta-analysis showed high heterogeneity (I^2=69%).[20] A meta-analysis of individual patient data from the same authors, with 3 additional trials,[46-48] found that the use of LMWH did not have an effect on prevention of placental complications, regardless of thrombophilia, concomitant LDA use, timing of treatment or dosage of LMWH.[21] An imprecise classification of the disease, together with the fact that PE and other placental complications such as SGA are multifactorial conditions, could explain inconsistency among studies. These authors recognized the need for multicenter clinical studies for further research and confirmation. Recently, a number of new randomized trials have been published (HEPEPE,[38] EPPI[22], Karadag et al.,[39] FRUIT-II.,[45] and HOPPE[24] trials). In our meta-analysis, a total of 15 studies (2848 participants) were reviewed. In high-risk women, treatment with LMWH compared with standard treatment (including LDA) was associated with significant reduction in development of PE; compared with previous meta-analysis, we had a greater sample size to analyze and a unique main outcome (PE). Our analysis also included 4 older studies[34, 40, 42, 44] not previously analyzed in other reviews. Considering the previous conflicting findings on efficacy of LMWH for prevention of PE, and that these could be explained by association with LDA, heterogeneity of research population, type of

LMWH, timing of treatment and study-center effects, we decided to perform sub-analyses taking into account these different aspects.

Combination of LMWH and LDA has been prescribed empirically and been widely used in clinical practice for high-risk women, although their effectiveness has only been shown for obstetric antiphospholipid syndrome and suggested for second-trimester pregnancy loss. It is endorsed by the American College of Chest Physicians and the American College of Obstetrics and Gynecology for this indication.[49] The effectiveness of this combination may indeed be biologically plausible.[25, 50] However, to date, there is limited evidence that the combination of antithrombotic drugs is efficacious in preventing recurrence of PE. Sub-analysis according to concomitant use of LDA was performed, and data from 7 trials including 1092 women, showed that patients treated with LMWH combined with LDA presented significantly less PE than those treated with LDA alone. In fact, no benefit was observed when LMWH was administrated by itself. These findings are in line with a recent meta-analysis[23] involving 8 studies, which evaluated whether the combination of LMWH and LDA was superior to LDA alone in the prevention of PE or miscarriage. Criteria for recruitment was previous recurrent miscarriage in 5 studies (3 of which included women with thrombophilia), and a history of previous early-onset PE in 3 studies (1 including women with thrombophilia). While combination of prophylactic treatments was not beneficial in women with recurrent miscarriage, there was a reduction in PE and SGA neonates in women with previous PE, but this outcome only was evaluated in 3 of the 8 trials, that included 590 women. In the present meta-analysis, we obtained a wider sample size (1092 women) and with different inclusion criteria: pregnant women with a history of PE or SGA[22, 35, 37, 38, 41-43, 45], thrombophilia[39, 45, 46], recurrent miscarriage with and without thrombophilia[34, 39, 44, 46] and

women with previous abruption without thrombophilia[36]. On the basis of these results, in women with previous adverse obstetric history, combined treatment reduced not only PE and SGA, but also, perinatal death.

The beneficial effects of both LMWH and LDA on trophoblast invasion are well documented. Aspirin alone inhibits the production of both thromboxane and prostacyclin in trophoblast cultures[51] and may have an antiapoptotic effect.[52] However, aspirin alone has not been shown to promote trophoblast migration,[53] a key process for trophoblast invasion. In contrast, LMWH seems to have an effect on trophoblast invasion[53] but LMWH increases the release of anti-angiogenic factors and some pro-inflammatory molecules.[54, 55] An *in vitro* study that evaluated the action of aspirin and LMWH in trophoblast function, showed that their combination may counteract some of the adverse effects of LMWH when administered alone, potentially increasing their efficacy.[53]

Regarding secondary outcomes, LMWH was associated with a significant reduction in development of SGA, miscarriage; preterm delivery, and perinatal death. Regarding SGA, data extracted from the Rodger individual patient meta-analysis, showed a reduction in SGA defined below 10th centile and less than 5th centile, but not 3rd centile.[22] Moreover, LDA modestly reduced SGA in high-risk women.[56] In the present meta-analysis, the reduction of SGA was also more pronounced when LMWH was combined with LDA. For miscarriage, in the 8 RCTs that considered this outcome, patients treated with LMWH had significantly fewer miscarriages, however, only one trial[46] did not administer LDA in the control group, and its exclusion did not modify the results. Regarding preterm delivery, LMWH did not show significant benefit even when combined with LDA; only early administration before 16 weeks was significant, which may be indirectly

associated to prevention of early-PE. A reduction in perinatal death but not stillbirth, occurred among the offspring of LMWH-treated patients. The wide confidence intervals suggest a weak association due to ambiguous definitions and low frequency of this complication. Previous meta-analyses have not found any differences for pregnancy loss or neonatal death in patients treated with LMWH.[21]

The present meta-analysis also addresses the question of whether or not placenta-mediated pregnancy complications could be prevented by starting LMWH in the late first and early second trimesters of pregnancy. We performed subgroup analyses for this outcome, excluding the Kingdom et al.[40] and Rodger et al.[46] studies, which included patients that started treatment after 16 weeks of pregnancy, and we found that the impact of LMWH on PE and stillbirth was stronger. Below 16 weeks, the process of trophoblast invasion of the uterine spiral arteries is considered a crucial moment, when therapeutic measures still have the potential to improve maternal and perinatal outcomes.[12] Similarly, the positive effect of prophylactic LDA is also determined by gestational week at which treatment begins, and no benefit has been found when begun after 16 weeks.[57] We hypothesize that both LDA and LMWH have an impact on the physiological transformation of uterine spiral arteries, which is completed by 16 weeks' gestation.[58, 59]

Finally, no differences were observed between the treatment groups according to the presence of thrombophilia with respect to the incidence of PE or any other complication; in other words, there is no evidence that thrombophilia modifies the effect of heparin on the occurrence of any placental-mediated outcomes. In fact, the association between thrombophilia and placental complications is weak or absent for PE and SGA, the main outcomes of this study.[60, 61]

Interestingly, when we analyzed with regards to the type of heparin administrated, we observed that the majority of the described effects were found when enoxaparin was administrated, but not with dalteparin. Enoxaparin, dalteparin and tinzaparin differ in their mean anti-Xa activity and their ability to reduce thrombin generation.[62] Heparin dosage was administered according to maternal weight in most studies. This approach, appropriate for clinical practice, may also account for differences in their effects.

A sub-analysis for study-center effects showed that no effect on prevention of PE was observed in the analysis of the multicenter studies, whereas it remained significant when only unicenter studies were taken into account. The same holds true for the prevention of SGA. Single-center trials showed a larger heterogeneity compared to multicenter trials, that was also reported by Rodger et al.,[21] suggesting that publication bias, lower trial quality, and co-interventions could account for single-center positive results.

It is important to note that major side effects such as hemorrhage or PA were not increased in women treated with LMWH (with or without LDA). No statistically significant difference in bleeding was found between LMWH-treated and non-treated patients, regardless of whether or not it was combined with LDA. However, a recent register-based cohort study including 4088 women taking LDA during pregnancy, found that compared to women without LDA intake, there had been a higher incidence of intrapartum bleeding, postpartum hemorrhage and postpartum hematoma, especially in vaginal deliveries.[63] Information regarding definition and type of hemorrhage in the studies of the present meta-analysis was insufficient and therefore this information should also be taken cautiously. On the other hand, no differences in the occurrence of PA was found between groups; however, pathological confirmation was not

obtained in any of these studies. With regards to allergic or injection-site reactions, more episodes occurred in patients treated with LMWH, however other adverse effects were very rare.

Strengths and limitations

Strengths of our study include the inclusion of a larger number of studies and patients than other meta-analysis, as well as subgroup analyses by heparin type, which were not performed previously. In addition, 8 out of 15 of the studies included women with thrombophilia and we were able to evaluate the impact of this condition in treatment benefits, observing that the results were not affected by its presence. Even though variability of the inclusion criteria may contribute to heterogeneity in the results, it may represent a greater applicability of our findings both in patients with previous PE or SGA, and in patients with recurrent miscarriage (external validation). A final strength was that we were able to evaluate the side effects of LMWH, particularly the risk of PA and hemorrhage, especially when combined treatment with LDA was administrated, which is important information to take into account for clinical practice.

The main limitation of this meta-analysis is that methodological quality of the studies ranged from moderate to very low due to concerns about risk of bias (blinding was not possible in most trials, because of the nature of the intervention, i.e. subcutaneous injection). Additionally, the compliance of prescribed treatments was uncertain, with many women withdrawing their consent of participation. We are aware that some of the subgroups included patients with rare outcomes and these analyses were restricted by small samples. There were also lack of detail about important methodological issues in some studies, substantial heterogeneity detected in the analyses, and wide confidence intervals indicating imprecision in the results. Another limitation that one could argue is that heparin doses were not adjusted by

anti-factor Xa levels in any of the studies; however it has been reported that enoxaparin dosage adjusted according to anti-factor Xa levels compared to a fixed dose does not reduce the risk of recurrence of placental complications in women with thrombophilia.[64]

CONCLUSIONS and IMPLICATIONS

The clinical impact of our findings is important because prophylactic treatment with LDA is recommended in both *a priori* high-risk women (for previous adverse obstetrical outcomes) and in women screened by first trimester PE algorithms.[12] Although women that have already presented PE, SGA or fetal demise in a previous pregnancy account for only 15% of women developing preterm PE, they are at higher risk of recurrence.[65] The results of this meta-analysis suggest that high-risk women benefit from combination of LMWH and LDA in a synergic manner. Therefore, two different prevention strategies could be considered: 1) in *a priori* low-risk population screened for PE by first trimester algorithms, prophylaxis with 150 mg of aspirin daily from 12 weeks of gestation onward would be recommended;[12, 66] and 2) high-risk women based on adverse obstetric history of placental complications (early-onset PE, SGA or fetal demise) could benefit from a combined prophylaxis strategy with LMWH plus LDA begun before 16 weeks of gestation, regardless of thrombophilia; if such treatment is considered, the first choice would be enoxaparin.

In conclusion, LMWH reduces the incidence of PE and placenta-mediated complications in high-risk women with previous adverse obstetric history. However, the beneficial effects of LMWH depend on its combination with LDA. However, this meta-analysis gives rise to concerns regarding the low quality of available evidence and therefore, leads us to suggest that future trials examining the prevention of placental complications by LMWH in addition to LDA are

warranted before any clinical application is adopted. Based on the evidence available so far, we cannot recommend the use of LMWH alone in women at risk of placental complications.

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AUTHOR CONTRIBUTIONS

M. Cruz-Lemini: Record screening, assessment and evaluation of full-text articles, methodological quality assessment, writing original draft preparation, reviewing, editing and figure preparation. **E. Llurba:** Conceptualization, supervision, assessment and evaluation of full-text articles, methodological quality assessment, writing original draft preparation, reviewing, editing. ***JC. Vázquez:*** Databases search, methodology supervision, methodological quality assessment, software and data pooling and analysis, reviewing, editing and figure preparation. **J. Ullmo**: Assessment and evaluation of full-text articles, methodological quality assessment, reviewing, editing and figure preparation.

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TABLES

Table 1. Characteristics of studies included in this systematic review and meta-analysis.

First author, year (country)	Acronym	Study population	Interventions (sample size)	GA at inclusion	LMWH (dose)	Concomitant use of aspirin	Outcomes	Main findings
Badawy, 2008 (Egypt)	-	Women with a history of recurrent first trimester spontaneous abortion or miscarriage with no thrombophilia or antiphospholipid antibodies.	*LMWH + 0.5mg folic acid (n=170) *0.5mg folic acid (n=170)	<8 weeks	Enoxaparin 20mg (2000 IU) daily	Not reported (probable)	PE and PA reported as secondary outcomes.	No differences between both groups as regards the occurrence of PA and PA.
de Vries, 2012 (Netherlands)	FRUIT-RCT	Women with history of hypertensive disease and/or SGA <34 weeks, with inheritable thrombophilia, excluding antiphospholipid antibodies.	*LMWH + LDA (n=70) *LDA (n=69)	<12 weeks	Dalteparin 5000 IU daily (<50Kg 2500UI, >80Kg 7500 UI)	Yes; 75mg, 80mg or 100mg daily in all women	Recurrent PE, HELLP syndrome or eclampsia, and/or SGA.	Combined LMWH and LDA reduces recurrent hypertensive disease and/or SGA <34 weeks in women with inheritable thrombophilia.
Gris, 2010 (France)	NOH-AP	Women with history of PA but no fetal loss and no antiphospholipid antibodies.	*Standard high-risk care + LMWH (n=80)	At positive pregnancy test (mean,	Enoxaparin 40mg (4000 IU) daily	Yes; 100mg daily. 41 control and 19 treatment	Composite of PE, PA, SGA <5th percentile and/or stillbirth.	Enoxaparin lowers the risk of developing PE, PA, SGA <5th percentile and/or stillbirth

			*Standard high-risk care (n=80)	6 weeks)		women received LDA		in women with history of PA.
Gris, 2011 (France)	NOH-PE	Women with history of severe PE but no fetal loss and no antiphospholipid antibodies.	*LMWH + LDA (n=112) *LDA (n=112)	At positive pregnancy test (mean, 6 weeks)	Enoxaparin 40mg (4000 IU) daily	100mg daily in all women	Composite of PE, PA, SGA <5th percentile and/or stillbirth.	Enoxaparin lowers the risk of developing PE, PA, SGA <5th percentile and/or stillbirth in women with history of severe PE.
Groom, 2017 (New Zealand)	EPPI	Women at high risk of PE and/or SGA based on their obstetric history.	*Standard high-risk care + LMWH (n=73) *Standard high-risk care (n=83)	6-16 weeks	Enoxaparin 40mg (4000 IU) daily	100mg daily in all women	Composite of PE and/or SGA <5th percentile.	Enoxaparin in addition to standard high-risk care does not reduce the risk of recurrence of PE and SGA.
Haddad, 2016 (France)	HEPEPE	Women with history of severe PE diagnosed <34 weeks of gestation.	*LMWH + LDA (n=122) *LDA (n=122)	<14 weeks	Enoxaparin 40mg (4000 IU) daily	100mg daily in all women	Composite outcome (maternal death, perinatal death, PE, PA or SGA).	Enoxaparin does not significantly reduce placenta-mediated complications in women receiving LDA.
Karadağ, 2020 (Turkey)	-	Women with factor V Leiden mutation and	LMWH + LDA (n=59) *LDA	6 weeks	Enoxaparin 40mg (4000 IU) daily	Yes in 2 groups; 100mg daily	PE, HELLP syndrome, FGR, stillbirth, preterm birth	LMWH is more effective than aspirin alone in preventing PE.

		recurrent pregnancy loss.	(n=61) *LMWH (n=54)				and PA reported as outcomes.	
Kingdom, 2011 (Canada)	-	Women ay high-risk for placental dysfunction determined by biochemical marker or uterine Doppler screening.	*Standard high-risk care + LMWH (n=16) *Standard high-risk care (n=16)	18-23^{+6} weeks	Unfractionated heparin 7500 IU twice daily	8 control women received LDA	PE, eclampsia, HELLP syndrome, FGR, stillbirth and PA reported as secondary outcomes.	No statistically significant differences identified between the two treatment groups in the occurrence of all PE or severe PE.
Llurba, 2020 (Spain)	HOPPE	Women classified as high-risk based on medical history, and selected by first trimester screening of PE, without thrombophilia.	*LMWH (n=134) *No intervention (n=144)	<16 weeks	Enoxaparin 40mg (4000 IU), 60mg (6000 IU) >90Kg, daily	Yes; 100mg. 26 control and 20 treatment women received LDA	Composite outcome of PE, SGA, PA or stillbirth.	LMWH did not reduce the incidence of placenta-mediated complications.
Martinelli, 2012 (Italy)	HAPPY	Women with previous history of PE, HELLP syndrome, SGA, FGR, stillbirth or PA.	*Standard high-risk care + LMWH (n=63) *Standard high-risk care (n=65)	<12 weeks	Nadroparin 3800 IU daily	Yes; dose not specified	Composite end point of PE, eclampsia, HELLP syndrome, FGR, stillbirth or PA.	Nadroparin did not prevent late pregnancy complications in women at risk of recurrence.
Mello, 2005 (Italy)	-	Women with angiotensin-converting enzyme DD	*LMWH (n=41) *No intervention	<12 weeks	Dalteparin 5000 IU daily	Not reported	PE and FGR before 34 weeks.	LMWH reduces the recurrence of PE in ACE DD

		genotype non-thrombophilic women with history of preeclampsia.	(n=39)					homozygote women with a history of PE.
Rey, 2009 (Canada)	-	Women without thrombophilia and history of severe PE, SGA, stillbirth or PA.	*LMWH (n=58) *No intervention (n=58)	<16 weeks	Dalteparin 4000 IU <60Kg, 5000 IU 60-90Kg and 6000 IU >90Kg, daily	Yes; dose not specified. 46 control and 51 treatment women received LDA	Composite outcome (severe or early onset PE, SGA, PA or fetal death).	Dalteparin reduces recurrence of placental-mediated complications in women without thrombophilia.
Rodger, 2014 (Canada)	TIPPS	Women with thrombophilia at increased risk of venous thromboembolism, or with previous placenta-mediated pregnancy complications.	*LMWH (n=146) *Placebo/no intervention (n=143)	<21 weeks	Dalteparin 5000 IU daily <20 weeks, twice daily 20-37 weeks	Yes; dose not specified. 57 control and 43 treatment women received LDA	Composite outcome (severe or early onset PE, SGA, pregnancy loss, or venous thromboembolism).	Dalteparin does not reduce the occurrence of venous thromboembolism, pregnancy loss, or placenta-mediated pregnancy complications in pregnant women with thrombophilia.
Schleussner, 2015 (Germany)	ETHIG II	Women with at least 2 consecutive early miscarriages or 1 late miscarriage.	*Multivitamins + LMWH (n=226) *Multivitamins (n=223)	5-8 weeks	Dalteparin 5000 IU daily	Yes; 100mg daily. 13 control and 7 treatment women	Incidence of PE or HELLP syndrome reported as secondary outcome.	LMWH does not increase ongoing pregnancy or live-birth rates. Trial underpowered to clarify

						received LDA		whether LMWH may reduce placenta-mediated pregnancy complications.
van Hoorn, 2016 (Netherlands)	FRUIT-RCT II	Women with antiphospholipid antibodies and previous delivery for hypertensive disease and/or SGA <34 weeks.	*LMWH + LDA (n=16) *LDA (n=16)	<12 weeks	Dalteparin 5000 IU daily (<50Kg 2500UI, >80Kg 7500 UI)	Yes; 80mg or 100mg daily	Hypertensive disease: PE and/or eclampsia and/or HELLP syndrome <34 weeks.	Combined LMWH and LDA did not show reduction of recurrent hypertensive disease, compared with LDA alone.
Studies shown in alphabetical order. GA, gestational age; LMWH, low-molecular-weight heparin; LDA, low-dose aspirin; PE, preeclampsia; SGA, small-for-gestational age; FGR, fetal growth restriction; PA, placental abruption; HELLP, hemolysis, elevated liver enzymes and low platelet syndrome.								

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FIGURE LEGENDS

Figure 1. PRISMA flow chart: Summary of evidence search and selection.

PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

Figure 2. Effect of low-molecular-weight heparin on prevention of preeclampsia, alone and in combination with low-dose aspirin.

Figure 3. Effect of low-molecular-weight heparin on prevention of early preeclampsia (<34 weeks).

Figure 4. Effect of low-molecular-weight heparin on prevention of preeclampsia started before 16 weeks of gestation.

Figure 5. Effect of low-molecular-weight heparin on prevention of preeclampsia adjusted by the presence of thrombophilia.

Figure 6. Effect of type of low-molecular-weight heparin administered on prevention of preeclampsia.

Figure 7. Effect of low-molecular-weight heparin on prevention of SGA, alone and in combination with low-dose aspirin.

Figure 8. Bleeding or hemorrhage associated to treatment with low-molecular-weight heparin.

Figure 9. Treatment-related allergies associated to treatment with low-molecular-weight heparin.