Protocol:

Low-dose aspirin (LDA), low-molecular weight (LMWH) or their combination for the prevention of preeclampsia or small for gestational age fetuses in high-risk women: a network meta-analysis

Alexandros Sotiriadis
Anna Chaimani
Alexandra Tsiami
George Makrydimas
Makarios Eleftheriades
Efstratios Assimakopoulos
Basky Thilaganathan
Background

The prevention of preeclampsia has been one of the major objectives in Maternal-Fetal Medicine. While its traditional history-based prediction has been ineffective, recent algorithms achieve quite a high prediction rate at the first trimester\(^1\). A meta-analysis from 2010 highlighted the potential of low-dose aspirin (LDA) to reduce the risk for preeclampsia and fetal growth restriction (FGR), and even more so of its early and severe form, when commenced before 16 weeks\(^2,4\). This much-anticipated news quickly received a welcome reception, and many scientific bodies, including the American College of Obstetricians and Gynecologists\(^5\) and the British National Institute of Health and Care Excellence\(^6\), currently recommend administration of low-dose (60-80 mg) aspirin in women at high risk for PE, starting in the first trimester. Recently, the same group which authored the 2010 meta-analysis publishes a new meta-analysis comparing the effectiveness of combined LDA plus low molecular weight heparin (LMWH) to LDA alone for the prevention of preeclampsia\(^7\). Pooling the results of three studies, the authors found that, in women with previous preeclampsia, the combination of LDA+LMWH is more effective than LDA alone (RR 0.54, 95% CI 0.31-0.92)\(^7\). These results may spark a new discussion, especially at a time when the large ASPRE trial on LDA (https://fetalmedicine.org/aspre-1) is ongoing.

The main limitations of the existing meta-analyses are, first, that they are based on evidence of suboptimal quality, and, second, that the magnitude of this evidence is limited anyway. Given that the first limitation cannot be overcome with the existing studies, we will perform a network meta-analysis in order to optimize the utilization of the existing published data, which may address the latter problem.

Description of the condition

According to the current ACOG guidance, preeclampsia is primarily defined by the occurrence of new-onset hypertension (i.e. systolic BP $\geq$140 mm Hg, or diastolic BP $\geq$90 mm Hg, or both) plus proteinuria (i.e. excretion of $\geq$300 mg of protein per 24-h urine) (http://www.acog.org/Resources-And-Publications/Task-Force-and-Work-Group-Reports/Hypertension-in-Pregnancy)

Fetal growth restriction is actually the failure of a fetus to reach its intrauterine developmental potential. As this is often difficult to calculate in practice, a small-for-gestational age fetus (SGA, defined as estimated fetal weight <10th, 5th or 3rd centile) is used as a proxy. In the present meta-analysis we will use SGA, defined as estimated fetal weight <10 the centile for gestational age, as an outcome.

Description of the interventions

Several strategies have been tried for the prevention of preeclampsia and most of them have proven ineffective. Antioxidants (i.e. vitamin C or vitamin E, alone or in combination with other supplements) were not found to be more effective than placebo\(^8,9\). On the contrary, calcium supplementation
appeared to lower the risk for preeclampsia in women with low dietary intake\textsuperscript{10,11}. However, the most abundant evidence regards antiplatelet agents, essentially low-dose aspirin, which was found to be effective in reducing the risk for preeclampsia and intrauterine growth restriction when commenced before 16 weeks\textsuperscript{2}. Recently, a meta-analysis indicated that the combination of LDA+LMWH may be superior to LDA alone\textsuperscript{12}. In clinical practice, extrapolating the knowledge from basic research and the experience from thrombophilias, the most commonly used agents include LDA, low-molecular-weight heparin (LMWH) or LDA_LMWH, and this network meta-analysis will focus on these.

**How the intervention might work**

Unfractioned- and low molecular weight (LMWH) heparin and low-dose aspirin (LDA) have been tried for a long time and, although their effectiveness is only proven for cases with obstetric antiphospholipid syndrome\textsuperscript{13,14} and possibly second-trimester pregnancy loss\textsuperscript{15}, their use is currently widespread in a multitude of cases deemed at high risk for poor perinatal outcome.

Both LDA and LMWH are mostly known for their anticoagulant effects, but there is ample experimental evidence indicating that both drugs exhibit direct actions on the developing trophoblast.

Normally, there is an equilibrium in the trophoblastic production of thromboxane and prostacycline. This equilibrium is distorted in preeclamptic placentas, where the release of thromboxane is significantly increased while the release of prostacyclin is significantly decreased\textsuperscript{16}. Aspirin has been shown to inhibit the production of both thromboxane and prostacyclin in trophoblast cultures, and this effect is particularly pronounced for thromboxane in preeclamptic women\textsuperscript{17}. Aspirin may also have antiapoptotic effect on the trophoblast, as the addition of aspirin (or heparin for that matter) in cultured BeWo cells (a choriocarcinoma cell line that can serve as an experimental model for preeclampsia\textsuperscript{18}) treated with sera from failed IVF cases decreased their apoptosis\textsuperscript{19}. Therefore, aspirin may be associated with attenuation of nitric oxide synthase activity and potentially prevent oxidative stress and apoptosis.

Similar to aspirin, heparin has also exhibited favorable effects on trophoblast in vitro, apart from its anticoagulant action. A study on cultured human first-trimester villous trophoblast showed that treatment with unfractioned heparin significantly increased the number of viable cells in a dose-dependent manner and reduced apoptosis by ≥50%\textsuperscript{20}. In addition, incubation with enoxaparin induced the expression of matrix metalloproteinase (MMP)-9 in human extravillous trophoblast (EVT) cells and eventually enhanced their invasiveness on matrigel by 40-80\%\textsuperscript{21}. In a similar study, both enoxaparin and (particularly) tinzaparin increased EVT cells invasiveness on matrigel by promoting the activity of MMP-2 and stimulating the expression of heparin binding-EGF (HB-EGF) and cystein-rich angiogenic inducer 61 (Cyr61), which are both molecules that support trophoblast invasion and migration\textsuperscript{22}. However, the findings in favor of heparin are not unanimous and there are data that in fact indicate an adverse effect of heparin on invasion. Thus, an in vitro study on a human extravillous trophoblast line (SGHPL-4) showed that addition of either unfractioned- or low molecular weight heparin significantly suppressed hepatocyte growth factor (HGF)-stimulated invasion, without affecting the invasion of unstimulated SGHPL-4 cells\textsuperscript{23}. 
In addition, there is evidence that heparin increases the release of the antiangiogenic soluble fms-like tyrosine kinase-1 (sFlt-1) and impairs signaling of vascular endothelial growth factor (VEGF), possibly exerting a deleterious effect on the developing placenta. An in vitro study in floating villi from placentas of normal and complicated pregnancies showed that dalteparin sodium increased sFlt-1 levels, both by releasing previously bound sFlt-1 and by increasing its expression. As a counteract, dalteparin treatment also resulted in increased release of the pro-angiogenic placental growth factor (PIGF), whereas soluble endoglin (sENG) release remained unaltered and VEGF was undetected. Overall, the net effect of dalteparin was considered to be antiangiogenic and may antagonise the actions of VEGF and PIGF on the maternal endothelium. Interestingly, the same study indicated that lower levels of dalteparin increased syncytial fusion, differentiation and turnover, whereas supra-physiological levels favored syncytiotrophoblast necrosis. These effects have also been observed in vivo: a study in 11 preeclamptic women at the third trimester showed that administration of enoxaparin resulted in a 26% mean increase in serum sFlt-1 levels two hours post-injection, the magnitude of this effect being dependent on the initial sFlt-1 levels. Similar to the previous study, this antiangiogenic effect was partially counterbalanced by a smaller (15% on average) increase in PIGF levels, resulting in relative constant ratios between the two concentrations.

A dual action of heparin may account for its apparently contradictory effects on the placenta. Indeed, a study on a human EVT cell line (HTR-8) showed that enoxaparin can have both pro- and anti-inflammatory effects: although it does not affect the basal levels of interleucin (IL)-6, IL-8 and IL-1β release, it partially reverts the antiphospholipid antibody-mediated elevations in IL-8 and IL-1β. Furthermore, the enoxaparin-related increase in the pro-inflammatory molecule GRO-a is negated with the addition of aspirin, indicating that the combination of the two drugs can counteract the pro-inflammatory effects of LMWH alone.

**Why it is important to do this review**

Both common reasons that prompt conducting a network meta-analysis are present in this field, i.e.:

1. **Availability of many, pairwise independent comparisons for the treatments of interest, which however does not provide information about all comparison or the treatment hierarchy unless all information is synthesized in one step, and,**
2. **Absence of head-to-head trials for all or some of the treatments of interest which creates uncertainty for decision makers. For example, although LDA, LMWH and combined LDA+LMWH are widely used in clinical practice, there are only few studies comparing LDA to LDA+LMWH, which may increase uncertainty. Even more importantly, there are no studies directly comparing LDA to LMWH, LMWH to LDA+LMWH and LMWH to placebo.**

**Objectives**
The aim of this network meta-analysis is to compare the efficiency of LDA, LMWH, LDA+LMWH or placebo/no treatment in preventing preeclampsia or SGA in women at high risk for these conditions.

We also aim to generate a clinically meaningful hierarchy of interventions according to their ability to reduce the rates of preeclampsia and SGA.

Methods

Criteria for considering studies for this review

The specific criteria for considering studies will be as follows

Types of studies

Randomized controlled trials comparing low dose aspirin (LDA) or low molecular weight heparin (LMWH) or their combinations versus each other or placebo / no treatment prior to 16 gestational weeks for the prevention of preeclampsia (PE) or small-for –gestational age (SGA) infant in women at high risk for this complications. No language, country or publication dates restrictions will be imposed.

Types of participants

Inclusion criteria: women at high risk for preeclampsia or SGA (as defined by the authors of the primary studies), entering randomization before 16 gestational weeks.

Exclusion criteria: Randomization after 16 weeks; entry criteria for randomization other than high risk for preeclampsia or SGA, (e.g. general population, recurrent miscarriage or thrombophilia as the only entry criterion). Studies with antiphospholipid syndrome as the only entry criterion will also be excluded, as the combination LDA+LMWH is already the preferred prophylactic treatment for this syndrome 27.

Types of interventions

Either of the following: (1) Low-dose aspirin (LDA), as defined by the authors; (2) Low-molecular weight heparin (LMWH) (any agent and regimen); (3) combinations of LDA+LMWH, (4) placebo or no treatment. We will exclude from this network meta-analysis strategies that have been tried less rigorously or are not commonly used in current clinical practice (e.g. unfractioned heparin) or apply to specific subpopulations (e.g. supplements for malnurished women)We will merge different doses of aspirin under the “LDA” designation as well as different preparations and doses of LMWH. If sufficient data are available we will split the different variants of these intervention nodes in a subgroup analysis.

We believe that the assumption of joint randomizability is likely to be plausible in our data and we assume that any patient who meets the inclusion criteria is, in principle, equally likely to be randomized to any of the eligible interventions.
Types of outcome measures

• Primary outcomes:

1. Development of preeclampsia (any degree of preeclampsia developing at any gestational age, as defined by the authors) in the index pregnancy

2. Development of small for gestational age (SGA) fetus, (commonly defined as fetal growth <10th centile or lower)

• Secondary outcomes

1. Development of early preeclampsia (as defined by the authors) in the index pregnancy

2. Development of severe preeclampsia (as defined by the authors) in the index pregnancy

Search methods for identification of studies

Trials that compare at least two of the eligible interventions and meet all other inclusion criteria will be eligible.

Eligible studies will be identified by a predefined search strategy in electronic databases. We will search the literature for randomized clinical trials comparing any of the above interventions for the prevention of preeclampsia in women at high risk for the condition. Medline, Scopus, Cochrane Central Register of Controlled Trials (CENTRAL) and the U.S. Registry of clinical trials (www.clinicaltrials.gov) will be searched from inception using combinations of the terms “aspirin”, “heparin”, “dalteparin”, “enoxaparin”, “nadroparin”, “bemiparin”, “tinzaparin”, “reviparin”, “parnaparin”, “preeclampsia”, “growth restriction”, “growth retardation”, “fetal growth”, “small for gestational age”, “SGA”, “IUGR” The search terms are deliberately wide in order to minimize the potential to miss studies. These searches will be complemented by perusal of the references of the retrieved articles and additional automated search using PubMed’s “search for related articles” function. Previously published meta-analyses on the topic were also searched. All studies will be carefully compared to avoid inclusion of duplicate or overlapping samples. In case of overlap, the study with the largest number of cases will be included.

Data collection and analysis

The study characteristics of each included study will be assessed according to a predefined data extraction form, including inclusion and exclusion criteria, definitions for preeclampsia and SGA and treatment protocols.
Data extraction and management

Extracted data will be reported and stored in a dedicated spreadsheet containing all pertinent information.

Outcome data

All outcomes are dichotomous. The extracted data from each study for each of the primary and secondary outcomes will include (i) the definition of the outcome, (ii) the regimen for each intervention tested, (iii) the attrition rate per arm, (iv) the number of events and number of participants in each intervention arm for each study. Arm level data will be extracted.

Data on potential effect modifiers

From each included study we will extract data on the following study, intervention and population characteristics that may act as effect modifiers:

1. Entry criteria, i.e. how was “high risk” defined in each study
2. Exclusion criteria, given that, similarly to entry criteria, they tend to significantly vary across studies
3. Specific drug preparation, dose, gestational age at commencement
4. Definition of outcomes, i.e. criteria by which preeclampsia and SGA were defined in each study.

Assessment of risk of bias in included studies

The risk of bias in individual studies will be assessed using the Cochrane “risk of bias” tool\textsuperscript{28}. We will assess the following risk of bias items: random sequence generation, allocation concealment, blinding of participants, personnel and assessors, incomplete outcome data, selective reporting. Then, we will classify studies as being overall at low risk of bias when none of these items is rated at high risk and less than four at unclear risk, and at moderate risk of bias when one item is rated at high risk or none is rated at risk but four or more at unclear risk; in all other cases studies will be considered being overall at high risk of bias.

Measures of treatment effect

Relative treatment effects

For all possible pairwise comparisons, summary risk ratios (RRs) with 95% confidence intervals (CIs) will be estimated.

Relative treatment ranking
For each treatment, we will estimate the ranking probabilities of assuming any possible rank, plotted the cumulative ranking curves and calculated the surface under it (SUCRA). SUCRA is a percentage that shows how much effectiveness a treatment achieves in comparison with an imaginary treatment which is always the best without uncertainty. The largest the SUCRA value, the better the rank of the treatment 29,30.

**Unit of analysis issues**

It is unlikely to identify cross-over or cluster randomized studies for these outcomes. We will account for the inherent correlation introduced by multi-arm trials.

**Assessment of clinical and methodological heterogeneity within treatment comparisons**

We will assess the presence of clinical heterogeneity within each pairwise comparison by comparing the trial and study population characteristics across all eligible trials.

**Assessment of transitivity across treatment comparisons**

Details on patient and study characteristics that could act as effect modifiers and may introduce intransitivity will be recorded and described in a dedicated Table. We will assess statistically the potential for important intransitivity in the data by comparing the distribution of the potential effect modifiers (described in the section data extraction and management) across the available direct comparisons in the network 31,32. We will also assess the clinical comparability of the different drug preparations and doses before merging them into the same node.

**Data synthesis**

**Methods for direct treatment comparisons**

Standard meta-analysis will be initially performed for direct comparisons involving at least two studies. Direct estimates will be derived using for each outcome a comparison-specific random effects model in Open Meta-Analyst (http://www.cebm.brown.edu/open_meta/).

**Methods for indirect and mixed comparisons**

We will perform random effects network meta-analysis to compare simultaneously the relative effectiveness of all interventions 33 using the multivariate meta-analysis approach that treats the different comparisons in studies 34. We will run the analyses in Stata (StataCorp. 2011. Stata Statistical Software: Release 12, College Station, TX), using the network package 34 and the network graphs package 35.

**Assessment of statistical heterogeneity**

**Assumptions when estimating the heterogeneity**
We will assume a different heterogeneity parameter for each direct meta-analysis and a common heterogeneity across all comparisons within each outcome for the network meta-analysis.

**Measures and tests for heterogeneity**

The measures used for the assessment of heterogeneity in standard pairwise meta-analysis will be Q-test and I² statistic. For network meta-analysis we will compare the common heterogeneity τ with previously derived empirical distributions for heterogeneity 36.

Prediction intervals (PrI), which indicate the interval within which the relative effect of a future study is expected to be 37,38, will be estimated and their plots will be constructed in order to aid interpretation of the random effects meta-analysis. This will be done using the network graphs package in Stata. The prediction interval plot also gives information about the extent and impact of the common heterogeneity on each relative treatment effect 30.

**Assessment of statistical inconsistency**

We will assess the consistency of treatment effects, i.e. the agreement of direct and indirect evidence, by constructing an inconsistency plot, using the loop-specific approach 39. We will perform this approach assuming a common heterogeneity parameter across all loops in the network as derived from the network meta-analysis model. We will also run the design-by-treatment interaction model to assess the presence of inconsistency in the entire network 40.

**Sensitivity analyses**

We will assess the impact of risk of bias in the results by excluding studies being at overall high risk of bias in a sensitivity analysis.

Moreover, in order to assess the potential impact of different LDA doses, we will perform a sensitivity analysis breaking LDA to two nodes, i.e. <100 mg/d and 100 mg/d.
References

17. Cervar M, Nelson DM, Kainer F, Desoye G. Drug actions in preeclampsia: aspirin, but not magnesium chloride or dihydralazine, differentially inhibits cultured human trophoblast release of