Thromboprophylaxis for recurrent miscarriage in women with or without thrombophilia

HABENOX*: A randomised multicentre trial

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Summary
Recurrent miscarriage affects 1–2% of women. In more than half of all recurrent miscarriage the cause still remains uncertain. Thrombophilia has been identified in about 50% of women with recurrent miscarriage and thromboprophylaxis has been suggested as an option of treatment. A randomised double-blind (for aspirin) multicentre trial was performed among 207 women with three or more consecutive first trimester (<13 weeks) miscarriages, two or more second trimester (13–24 weeks) miscarriages or one third trimester fetal loss combined with one first trimester miscarriage. Women were analysed for thrombophilia. After complete work-up, women were randomly allocated before seven weeks’ gestation to either enoxaparin 40 mg and placebo (n=68), enoxaparin 40 mg and aspirin 100 mg (n=63) or aspirin 100 mg (n=76). The primary outcome was live-birth rate. Secondary outcomes were pregnancy complications, neonatal outcome and adverse effects. The trial was ended prematurely because of slow recruitment. A live birth rate of 71% [relative risk (RR) 1.17, 95% confidence interval (CI) 0.92–1.48] was found for enoxaparin and placebo and 65% [RR 1.08, 95% CI 0.83–1.39] for enoxaparin and aspirin when compared to aspirin alone (61%, reference group). In the whole study group the live birth rate was 65% (95% CI 58.66–71.74) for women with three or more miscarriages (n=204). No difference in pregnancy complications, neonatal outcome or adverse effects was observed. No significant difference in live birth rate was found with enoxaparin treatment versus aspirin or a combination of both versus aspirin in women with recurrent miscarriage.

Keywords
Pregnancy, clinical trials, heparins/LMWH, thrombophilia

Introduction
Recurrent miscarriage has commonly been defined as three or more consecutive spontaneous pregnancy losses (1). Approximately 10–15% of all conceptions end up in a miscarriage, which is mostly due to chromosomal abnormalities. About 1–2% of women suffer from recurrent miscarriage, having a major impact on their lives (2, 3). The cause appears to be multifactorial, such as uterine anomalies, endocrine disorders, immunological causes, infections, chromosomal abnormalities and maternal autoimmune disorders. However, in 50–60% of all recurrent miscarriage the cause remains unclear (4, 5).

In a large meta-analysis different thrombophilic polymorphisms have been identified to be associated with recurrent fetal loss. However, the association depended on type of thrombophilic disorder and type of fetal loss (6).

Subsequently, interventions with thromboprophylaxis for prevention of recurrent miscarriage have been proposed (7–9). The idea of using low-molecular-weight heparin (LMWH) and aspirin in women with recurrent miscarriage came from the positive effect found in women with antiphospholipid syndrome, which has also been associated with risk of thrombosis (10, 11). Nevertheless, insufficient evidence for the use of anticoagulants for women with unexplained pregnancy loss was found in two systematic reviews (12, 13). Additionally, LMWH has been identified, besides the anticoagulant mechanism, to have also an anti-inflammatory and immunomodulatory action (14–16). The positive effect in women with antiphospholipid syndrome might therefore rather be an anti-complement effect than an anticoagulation effect (17).

Very recently two placebo controlled randomised trials have found no beneficial effect of thromboprophylaxis for women with two or more miscarriages (18, 19). The aim of this multicentre ran-
domised controlled trial (RCT) was to compare aspirin and/or LMWH in women with recurrent miscarriage with or without thrombophilia.

**Materials and methods**

We performed a randomised controlled multicentre study in Helsinki University Hospital, and Oulu University Hospital, Finland; Leiden University Medical Centre, the Netherlands and Karolinska University Hospital, Sweden. The enrolment was performed from January 2002 until December 2007. The trial was approved by the Ethics Committee of all participating centres. Written informed consent was obtained from all patients prior to randomisation. At the start of the trial, registration was not yet necessary, therefore the trial was registered in 2009 in ClinicalTrials.gov, trial register number NCT0095962. The study was ended prematurely by the authors because of slow recruitment in six years.

**Participants**

Women aged 18–41 years with recurrent miscarriage with or without thrombophilia were assessed for eligibility after referral. Recurrent miscarriage was defined as three or more consecutive first trimester (<13 weeks) miscarriages, two or more second trimester miscarriages (13–24 weeks) or one third trimester fetal loss (>24 weeks) combined with at least one first trimester miscarriage. Thrombophilia testing was performed before pregnancy and defined as factor V Leiden mutation, prothrombin gene mutation, protein C deficiency (<0.73 IU/ml), protein S deficiency (< 0.57 IU/ml), high factor VIII (≥1.5 IU/ml), anticardiolipin antibodies (8–40 GPL) or beta-2 glycoprotein (>20 U/ml). Beta-2 glycoprotein was only tested in Finland (116 women). A second test was performed after 12 weeks, but before inclusion in the study, to confirm positive thrombophilia testing. Women with combined thrombophilia (two or more findings) or “high-risk” thrombophilia, defined as positive lupus anticoagulant, antithrombin deficiency, anticardiolipin antibodies > 40 GPL or homozygosity for factor V Leiden, were included in a separate study (Habenox III), in which they were randomised to aspirin 100 mg and enoxaparin 80 mg or enoxaparin 80 mg alone. Other exclusion criteria were history of thromboembolism or bleeding disorders, allergy to aspirin or enoxaparin, uterine anomalies, cervical insufficiency, untreated thyroid disease, poorly treated diabetes mellitus, parental chromosomal abnormalities and pregnancies achieved by assisted reproductive techniques.

**Study design**

Women with thrombophilia were enrolled in Habenox I, women without thrombophilia were enrolled in Habenox II, followed by identical study protocols. Randomisation was performed after a positive HCG urine test and before seven weeks’ gestation by computer in blocks of six patients and stratified by centre (4 centres) and history of early (<13 weeks’ gestation) or late miscarriage (≥13 weeks’ gestation). Patients were allocated to randomisation code numbers in chronological order. The allocation list was stored at an independent secretary and randomisation was performed with communication by telephone per centre. Information about the treatment each woman was given was stored in a sealed envelope, which was not opened until the study was completed, unless such information was necessary in case of severe complications or serious side effects. The study was double-blinded for aspirin. To ensure blindness for care provider and patient, tablets were placed in identical opaque capsules and stored in sealed identical containers.

Women were randomly allocated to three different intervention groups. All interventions were given once daily. The first group received enoxaparin 40 mg as subcutaneous injection and placebo orally. The second group received enoxaparin 40 mg as subcutaneous injection and aspirin 100 mg orally. The third group received aspirin 100 mg orally. Women randomised to enoxaparin were taught to self-inject the medication subcutaneously. After starting enoxaparin, platelets were checked at every visit for heparin-induced thrombocytopenia. At the first visit a physical examination was performed, body mass index (BMI) and blood pressure were checked. Follow-up was performed by their own obstetrician at 8, 10, 14, 18, 24, 28, 32 and 36 weeks of gestation. During all visits an ultrasound was performed and patients were asked for adverse effects and vaginal bleeding complications, which were filled out on a case report form (CRF). Aspirin and placebo were discontinued at 36 weeks gestation. Enoxaparin was continued until the first signs of labour. Women with thrombophilia restarted enoxaparin after delivery and continued until six weeks postpartum.

**Outcome measures**

Primary outcome was live birth rate, defined as live birth after 24 weeks gestation. Secondary outcomes were preeclampsia, abruptio placenta, premature delivery (24–37 weeks gestation), intrauterine growth restriction (birth weight <2 SD), adverse effects and vaginal bleeding complications, defined as first, second and third trimester blood loss and postpartum haemorrhage (>500 ml blood loss).

**Sample size calculation**

The sample size estimation was based on, at the start of the study, the only RCT on this subject by Rai et al. (11) showing that aspirin and unfractioned heparin improved the live birth rate in women with phospholipid antibodies from 42% to 71% compared to aspirin alone. Therefore, for an increase of 30% in live birth rate, a sample size of 90 patients per treatment group was required to re-
ject the null-hypothesis on a 5% level with a power of 80% and approximately 270 patients were required to enter the study.

Statistical analysis

Data were analysed according to the intention to treat principle. Habenox I and II were analysed together as study protocols were identical. No interim analysis was planned. A subanalysis was planned for thrombophilia versus no thrombophilia and for a history of early versus late miscarriages. Data were expressed as the mean ± standard deviation (SD) for continuous variables. Additionally ranges were given for maternal age, BMI and previous miscarriages. A median was given for number of previous miscarriages. An ANOVA test was performed for comparisons between means. Categorical variables were expressed as 95% confidence interval (CI), and the Chi square test was used to compare proportions. Statistical significance was defined as p<0.05. Live birth rates were additionally expressed as relative risks with associated 95% CI, with aspirin as the reference group. Statistical analyses were performed using SPSS version 16.0 (Statistical Package for Social Science; SPSS Inc., Chicago, IL, USA).

After primary analysis, subgroup analysis was performed to assess treatment effects between different categories of women. Women were classified into subgroups based on their characteristics at trial entry: thrombophilia (yes or no), maternal age (<35 or ≥35), number of previous miscarriages (<4 or ≥4), parity (primary, previous live birth versus secondary, no previous live birth) and history of early versus late miscarriage/fetal loss (<13 weeks versus ≥13 weeks gestation).

Results

A total of 219 women were assessed for eligibility after referral (Fig. 1). Twelve women with combined or “high-risk” thrombophilia were excluded and randomised in another trial (see above). We randomly assigned 76 women to aspirin, 63 women to enoxaparin and aspirin, and 68 women to enoxaparin and placebo.
enoxaparin and aspirin and 68 women to enoxaparin and placebo. One woman allocated to the aspirin group developed a pulmonary embolism at eight weeks of gestation. She had not been diagnosed with thrombophilia before pregnancy and was additionally treated with LMWH according to local protocol. Two women in the enoxaparin and aspirin group used no medication or discontinued taking their medication. The enoxaparin and placebo group and the enoxaparin and aspirin group both included a carrier woman of a chromosomal abnormality associated with recurrent miscarriage. In no case was it necessary to reveal the used medication before the end of the study and open the sealed envelope.

Baseline characteristics of the study participants are shown in Table 1. Women allocated to aspirin were found to have a significant higher BMI compared to the other two intervention groups (p=0.003). In the whole group the median number of miscarriages was 3 (range 2–10). Only three (1%) of the included women had a history of two second trimester miscarriages and 38% of the women were found to have a history of four or more consecutive miscarriages.

Live birth rate

A live birth rate of 71% [relative risk (RR) 1.17, 95% CI 0.92–1.48] was found for enoxaparin and placebo and 65% [RR 1.08, 95% CI 0.83–1.39] for enoxaparin and aspirin when compared to aspirin alone (61%, reference group) (see Table 2). Exclusion of the five protocol violations from analysis gave no significant difference in live birth rate. Exclusion of the three women with only two miscarriages gave a live birth rate of 65% (95% CI 58.66–71.74) for all women (n=204).

Most of the miscarriages during the study occurred in the first trimester (93%), and 28% even before seven weeks gestation. One monochorionic, diamniotic twin pregnancy in the enoxaparin and aspirin group developed a twin-to-twin syndrome. Despite laser therapy, the pregnancy was lost at 17 weeks gestation. Another late miscarriage was identified at 17 weeks gestation in this intervention group. Chromosomal analysis of this fetus showed no abnormalities. One pregnancy in the enoxaparin and placebo group was terminated at 15 weeks gestation because of aneuploidy, Turner’s

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Enoxaparin and placebo (n=68)</th>
<th>Enoxaparin and aspirin (n=63)</th>
<th>Aspirin (n=76)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>32.5 ± 4.29 (22–40)</td>
<td>31.6 ± 4.57 (22–41)</td>
<td>32.0 ± 4.47 (22–40)</td>
<td>0.45</td>
</tr>
<tr>
<td>Range ≥35 years</td>
<td>24 (35.3)</td>
<td>17 (27.0)</td>
<td>26 (34.2)</td>
<td></td>
</tr>
<tr>
<td>Body mass index (Kg/m²) Range</td>
<td>23.4 ± 3.71 (18–36)</td>
<td>23.1 ± 3.13 (18–33)</td>
<td>25.4 ± 5.38 (17–40)</td>
<td></td>
</tr>
<tr>
<td>Blood pressure (mmHg) Systolic</td>
<td>117±11.31 70 ± 9.15</td>
<td>114 ± 11.00 70 ± 6.08</td>
<td>116 ± 11.67 72 ± 10.22</td>
<td></td>
</tr>
<tr>
<td>Diastolic</td>
<td>3.9 ± 1.41 3 (2–10) 30 (44.1)</td>
<td>3.6 ± 0.90 3 (3–7) 28 (44.4)</td>
<td>3.9 ± 1.48 3 (2–9) 21 (27.6)</td>
<td></td>
</tr>
<tr>
<td>Maternal smoking</td>
<td>7 (10.3)</td>
<td>6 (9.7)</td>
<td>11 (15.9)</td>
<td></td>
</tr>
<tr>
<td>Previous miscarriages ≥4</td>
<td>17 (25.0)</td>
<td>15 (23.8)</td>
<td>19 (25.0)</td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>32 (47.1)</td>
<td>42 (66.7)</td>
<td>46 (60.5)</td>
<td></td>
</tr>
<tr>
<td>Nulliparous</td>
<td>35.8 ± 4.64</td>
<td>36.5 ± 4.07</td>
<td>36.8 ± 4.15</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Demographic and baseline characteristics. Values are mean ± SD or number (%). For maternal age, body mass index and previous miscarriages ranges were given. A median was given for previous miscarriages.

Table 2: Primary outcome. Values are number (%) or relative risk with associated 95% CI.
syndrome. In two other pregnancies in this intervention group intrauterine fetal demise was identified at 15 and 24 weeks gestation. Both fetuses had normal chromosomal analysis.

Subanalysis of the women with identified thrombophilia showed a live birth rate of 76% [RR 1.21, 95% CI 0.79–1.87] for enoxaparin and placebo and 60% [RR 0.95, 95% CI 0.56–1.63] for enoxaparin and aspirin when compared to aspirin alone (63%, reference group). A high live birth rate was found for women with primary recurrent miscarriages [81%, RR 1.25, 95% CI 0.95–1.63] and women with four or more recurrent miscarriages [73%, RR 1.71, 95% CI 1.00–2.93] receiving enoxaparin and placebo compared to aspirin (Table 3).

Pregnancy complications and neonatal outcome

For pregnancy complications and neonatal outcome see Table 4. One abruptio placentae occurred in the aspirin group for which an emergency caesarean section was performed at 34 weeks gestation. Additionally, no significant difference in preeclampsia, intrauterine growth restriction or premature labour was observed between the three groups. One neonate in the enoxaparin and aspirin group died of respiratory distress syndrome one week after birth. This neonate was born severely intrauterine growth restricted (680 grams) at 27 weeks gestation. Another neonate in this group was diagnosed with an imperforate anus. In the enoxaparin and placebo group one neonate was born with a gastroschizis and one with cystic fibrosis.

Adverse effects

For adverse effects see Table 5. No heparin-induced thrombocytopenia was observed during the trial and no allergies were reported. Minor vaginal bleedings were observed during the first, second and third trimester of the pregnancy in all three groups. No significant difference was found between the three groups. However more blood transfusions were needed in four women in the enoxaparin and placebo group, compared to none in the other two groups. Minor side-effects such as skin reactions at injection site, bruising, itching, and nose bleeding were mentioned by the women, but of the whole study-group only one woman discon-

Table 3: Subgroups. Live birth number and relative risk with associated 95% CI was calculated in all subgroups for enoxaparin and placebo intervention versus aspirin and for enoxaparin and aspirin intervention versus aspirin.

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Enoxaparin and placebo (n=48)</th>
<th>Enoxaparin and aspirin (n=41)</th>
<th>Aspirin (n=46)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombophilia</td>
<td></td>
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</tr>
<tr>
<td>Yes</td>
<td>13/17 1.21 (0.79–1.87)</td>
<td>9/15 0.95 (0.56–1.63)</td>
<td>12/19 1.00</td>
<td>0.83</td>
</tr>
<tr>
<td>No</td>
<td>35/51 1.15 (0.87–1.53)</td>
<td>32/48 1.05 (0.78–1.42)</td>
<td>34/57 1.00</td>
<td></td>
</tr>
<tr>
<td>History of miscarriages</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Early</td>
<td>36/51 1.16 (0.89–1.52)</td>
<td>36/55 1.08 (0.82–1.43)</td>
<td>37/61 1.00</td>
<td></td>
</tr>
<tr>
<td>Late</td>
<td>12/17 1.18 (0.70–1.97)</td>
<td>5/8 1.04 (0.53–2.05)</td>
<td>9/15 1.00</td>
<td></td>
</tr>
<tr>
<td>History of miscarriages</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>26/32 1.25 (0.95–1.63)</td>
<td>27/42 0.99 (0.72–1.34)</td>
<td>30/46 1.00</td>
<td></td>
</tr>
<tr>
<td>Secondary</td>
<td>22/36 1.15 (0.75–1.75)</td>
<td>14/21 1.25 (0.80–1.96)</td>
<td>16/30 1.00</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>&lt;35 years</td>
<td>32/44 1.17 (0.88–1.56)</td>
<td>31/46 1.09 (0.81–1.46)</td>
<td>31/50 1.00</td>
<td></td>
</tr>
<tr>
<td>≥35 years</td>
<td>16/24 1.16 (0.75–1.78)</td>
<td>10/17 0.72 (0.41–1.29)</td>
<td>15/26 1.00</td>
<td></td>
</tr>
<tr>
<td>Number of miscarriages</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;4</td>
<td>26/38 1.02 (0.77–1.35)</td>
<td>23/35 0.98 (0.72–1.32)</td>
<td>37/55 1.00</td>
<td></td>
</tr>
<tr>
<td>≥4</td>
<td>22/30 1.71 (1.00–2.93)</td>
<td>18/28 1.50 (0.85–2.64)</td>
<td>9/21 1.00</td>
<td></td>
</tr>
</tbody>
</table>

Table 4: Pregnancy complications and neonatal outcome. Values are mean ± SD or number (%). * NICU, neonatal intensive care unit.

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continued her mediation at 12 weeks of her ongoing gestation. During the trial no women switched to another type of LMWH because of side-effects.

Discussion

This study was conducted to identify which thromboprophylactic treatment is best to prescribe to women with recurrent miscarriage without known cause or with thrombophilia. Our study shows no significant difference in success rate for treatment with enoxaparin versus aspirin or a combination of both.

Unfortunately, due to slow patient flow, only 3/4 of the women needed were recruited despite of the six years enrolment. The study had to be closed prematurely, leading to small intervention groups and skewed randomisation. Additionally, the sample size calculation of our study was based on an increase of live birth rate of 30% for treatment with enoxaparin, leaving our study not being able to exclude a benefit of enoxaparin only treatment. In order to have an 80% power to detect an increase of 10% in live birth rate at the 95% confidence level, a trial would need to randomise 356 women per treatment group. A trial of this size would take an enormous effort, time and finance. Still, the Habenox trial included in comparison with the other seven RCTs on thromboprophylaxis (18–24) most cases of women with three or more early miscarriages with initiating treatment before seven weeks gestation. With all studies being underpowered for showing an absolute risk of 10%, conclusions about the ineffectiveness of the used interventions must be made with caution, as the problem might be the number of patients (18–24).

A limitation of our study was that it included no placebo intervention group. It is therefore difficult to predict the a priori chance of our patients to miscarry in the subsequent pregnancy. The generally assumed live birth rate of 75% comes from a prospective longitudinal observational study and is higher than found in any of our treatment groups (25). More recent studies have found live birth rates ranging from 57%–95% (18–21, 26). The large range found for live birth rate in women with recurrent miscarriage might be explained by the use of different definitions of recurrent miscarriage (2 versus 3 miscarriages). This becomes clear when comparing the live birth rates of the Habenox trial with two other very recently published trials (Table 6) (18, 19).

Subgroup analysis showed a high (76%) but not significant live birth rate for women with thrombophilia treated with enoxaparin-only. This subgroup (n=51) was unfortunately very small as surprisingly we only tested 25% of the women positive for thrombophilia, and only 13% were found carrier of heritable thrombophilia. This was although comparable to the 16% found for inherited thrombophilia in the ALIFE trial (19).

In our study more obese women included in the aspirin group could have had an influence on the success rate (27). In recurrent miscarriage patients, Metwally et al. identified women with a BMI of >30 kg/m² to have a small but significant increased risk of miscarriage in the subsequent pregnancy (28).

The use of thromboprophylaxis during pregnancy appears to be safe for the mother and child as was found in a large systematic review (29). Our study however found an increase in the risk of postpartum blood loss and more blood transfusions in the enoxaparin and placebo group, this increased risk was not seen in the enoxaparin and aspirin group. This leads us to assume that it could be caused by chance and not treatment dependently.

<table>
<thead>
<tr>
<th>Table 5: Bleeding complications. Values are mean ± SD or number (%). * cc, cubic centimetre.</th>
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<tbody>
<tr>
<td><strong>Treatment</strong></td>
</tr>
<tr>
<td>Enoxaparin and placebo (n=48)</td>
</tr>
<tr>
<td>Enoxaparin and aspirin (n=41)</td>
</tr>
<tr>
<td>Aspirin (n=46)</td>
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<table>
<thead>
<tr>
<th>Table 6: Recent RCTs. *itt, intention-to-treat population.</th>
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<tbody>
<tr>
<td><strong>Treatment</strong></td>
</tr>
<tr>
<td>ALIFE 364 itt*</td>
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<tr>
<td>HABENOX 207</td>
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<tr>
<td>SPIN 294</td>
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</table>
In conclusion, no significant difference in live birth rate was found with enoxaparin treatment versus aspirin or a combination of both. All RCTs so far failed to reach enough power for showing an absolute risk of 10%, to exclude a possible effect of LMWH. Therefore an individual patient data meta-analysis might be the only possibility to provide us with evidence-based advice for the more homogenous group of women with three or more miscarriages or women with thrombophilia.

Acknowledgements
We would like to thank Eija Kortelainen for her assistance with data collecting.

Disclosures
The study was supported by an unrestricted grant from Sanofi-aventis for paying the salary of the study nurse (Finland), providing the randomisation list and aspirin and placebo medications. This funding source had no role in the study design, collection, analyses, and interpretation of data, writing of the report or the decision to submit the paper for publication. The corresponding author had full access to all data and had final responsibility for the decision to submit for publication.

What is known about this topic?
- Thromboprophylaxis prevents recurrent miscarriage in women with antiphospholipid syndrome.
- LMWH and/or aspirin did not improve live birth rate compared to placebo in the group of women with two or more early miscarriages.

What does this paper add?
- LMWH did not improve live birth rate compared to aspirin in women with three or more miscarriages.
- The Habenox study found a live birth rate of 65% (95% CI 58.66–71.74) for women with three or more miscarriages (n=204) on treatment.

References