Hypertensive Disorders of Pregnancy

Aspects of
Prevention and Follow-up

Carolien Nienke Heleen Abheiden

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Hypertensive Disorders of Pregnancy

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Prevention and Follow-up

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General Introduction

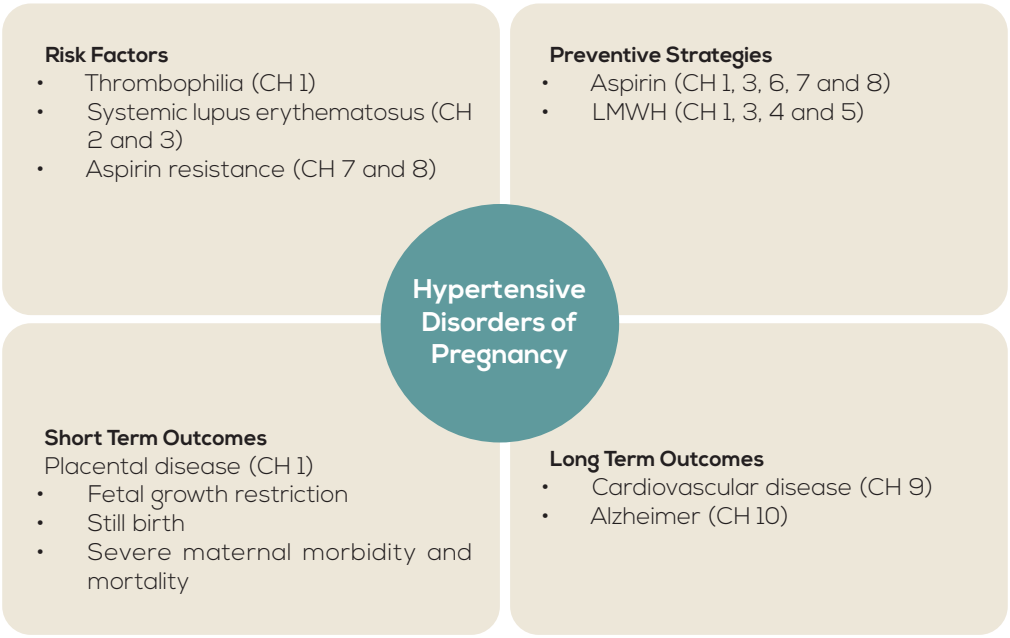
General introduction

A woman visits the obstetric outpatient clinic for her postpartum check-up, six weeks after the delivery of her second child. During her first pregnancy, she developed preeclampsia at 26 weeks gestational age. After admission at 25 weeks gestation due to fetal growth restriction (FGR) and abnormal uterine and umbilical flow velocity, her condition worsened and she was transferred to the intensive care unit due to multiple organ failure. After a caesarean section at 26 weeks and 2 days, the preeclampsia dissolved and she could be discharged after two weeks. The baby was admitted to the neonatal intensive care unit and could be discharged after ten weeks. Postpartum, thrombophilia examination revealed no inheritable or acquired anomalies. During her second pregnancy, aspirin was prescribed from eight weeks gestational age onwards. Preeclampsia recurred at 31 weeks gestational age and she underwent a caesarean section at 32 weeks gestational age. At the postpartum check-up, a few questions remained unanswered; what are reasons why aspirin does not prevent recurrent hypertensive disorders of pregnancy (HD) in every women? Could low-molecular-weight heparin (LMWH) be of additional value to prevent recurrent HD in absence of proven thrombophilia? What are the future cardio- and cerebrovascular health risks after a history of recurrent HD? This thesis contributes to the knowledge of anticoagulant and antiplatelet treatment options during pregnancy in several populations. Moreover, cardiovascular risk factors in women with thrombophilia after HD is examined, as well as a relation between HD and Alzheimer's disease.

Under the term HD, various expressions from mild to severe can occur and are defined as pregnancy induced hypertension (PIH): de-novo development of hypertension, preeclampsia: de-novo development of hypertension and proteinuria (>0.3g/24 hours), HELLP syndrome: thrombocytopenia (<100*109 /L), aspartate aminotransferase ≥ 70 IU/L and lactate dehydrogenase ≥ 600 IU/LHD and eclampsia: tonic-clonic seizures in a pregnant or recently delivered woman that cannot be attributed to epilepsy.[1] Nowadays, the definition of HD, especially concerning preeclampsia, has been expanded.[2;3] This thesis used the criteria according to Steegers et al,[1] and we focused on the more severe forms of HD, excluding PIH.
At present, HD is the most common cause of direct maternal mortality in the Netherlands.[4-6] In the Netherlands about 8% of all pregnant women (± 170.000 births per year) develop HD during pregnancy (including PIH), which is comparable to other developed countries.[7-9]
The exact pathophysiology of HD is not fully elucidated. HD seems to comprise different disease entities, but all are characterized by disturbed development of the placenta. The abnormal placentation induces systemic inflammatory and oxidative stress processes in the mother. In severe HD this results in endothelial damage in various organs.[3] The large impact of HD on women and their partners is well represented by the Dutch patient organization, 'stichting HELLP syndroom', providing medical information and personal support.

Four aspects are discussed in more detail in this general introduction to understand the set-up of this thesis; risk factors, preventive strategies, short- and long-term outcomes of HD (Figure 1).

Figure 1: Subjects in this thesis



1. Risk factors

Advanced maternal age, nulliparity, multiple pregnancy, preeclampsia in a previous pregnancy, BMI > 35 and a family history of pre-eclampsia (mother or sister) increase the risk to develop HD.[3] Moreover, maternal diseases like chronic hypertension, thrombophilia and systemic lupus erythematosus (SLE) increase risk for pregnancy complications including HD.

Thrombophilia

Inheritable thrombophilia increases the risk to develop a thromboembolic event. The most frequent types of inheritable thrombophilia in the Caucasian population are: factor V Leiden mutation (heterozygous 3-7%), prothrombin gene mutation (0.7-4%), deficiency in protein S (0.03-0.13%), protein C (+/-0.2%) and antithrombin (0-0.2%).[10] Some studies, including meta-analyses, show an association between inheritable thrombophilia and severe pregnancy complications like early-onset HD (HD < 34 weeks gestation) and small-for-gestational age (SGA) infants.[11-15] However, other studies did not support this finding.[16-18]

Acquired thrombophilia, including antiphospholipid syndrome (APS), is a non-genetic condition that increases the risk for thromboembolic events. APS can occur isolated: primary APS, or in relation to auto-immune diseases like SLE: secondary APS.[19] APS is considered to be present in a patient when at least one of the antiphospholipid antibodies (aPL; lupus anticoagulant (LAC), anticardiolipin antibodies (aCL) or β2-glycoprotein antibodies) is repeatedly measured in a blood

sample and at least one clinical criterion is present.[20,21] Clinical criteria for thrombotic APS include a history of thrombosis, criteria for obstetric APS include a history of pregnancy complications; intrauterine fetal death (IUFD), HD combined with birth <34 weeks gestation or more than two consecutive spontaneous miscarriages.[20,21] The actual prevalence of APS is not clear, since in daily practice not all people with thrombotic or obstetric events are tested for aPL. Women with APS have an increased risk to develop pregnancy complications like HD, FGR and IUFD.[22]

Systemic lupus erythematosus

SLE is an auto-immune disease that especially affects women during their childbearing age.[23] The prevalence of SLE varies between 7 and 160/100.000, with the highest prevalences in the non-Caucasian population. Nephritis, cutaneous disease, arthritis and thrombocytopenia are the most common expressions of SLE.[8,24] Optimal conditions for women with SLE to get pregnant include remission of SLE for at least six months.[25] SLE patients have a higher risk for HD, preterm birth, SGA and IUFD compared to women without SLE.[26,27] The presence of secondary APS further enlarges the risks of pregnancy complications.[28]

Aspirin resistance

Aspirin is considered as one of the main preventive strategies to reduce recurrence of HD.[29] Its mechanism of action will be discussed in the section about preventive strategies below. Aspirin resistance, however, could be a risk factor in the development of HD, since despite the use and timely start of aspirin, HD still occurs/recurs. After the publication of the FRactionated heparin in pregnant women with a history of Utero-placental Insufficiency and Thrombophilia trial (FRUIT-RCT),[30] Bujold et al. wrote a letter-to-the editor and suggested that recurrence of HD in the FRUIT-RCT might be caused by aspirin resistance.[31] Terms like laboratorial aspirin non-responders are used increasingly. We chose to use the term aspirin resistance because it is still the most well know term. There is neither a definition for aspirin resistance, nor a golden standard to measure it.[32] It has been described that aspirin resistance is associated with the development of new ischemic stroke, moreover, with increased clinical severity and stroke infarct volume.[33,34] Patients identified as being aspirin resistant could also have an increased risk for a major adverse cardiovascular event.[35] The phenomenon aspirin resistance in relation to pregnancy has been described in three other studies and one review.[36-39] About one third of the pregnant women seem to be resistant to aspirin. However, it is unknown yet whether aspirin resistance is the explanation for the recurrence of HD.

2. Preventive strategies

Nowadays, the prescription of low-dose aspirin and/or LMWH is still debated for most pregnancy complications. It is commonly accepted that prescription of aspirin to women with a history of HD and SGA infants is benificial.[29,40] The number of studies investigating the effect of LMWH in pregnancy is still limited. For women with inheritable thrombophilia, APS and SLE the current advises for aspirin and LMWH use during pregnancy differ per country

(Table 1). In women with inheritable thrombophilia and a history of HD or a SGA-infant and delivery before 34 weeks gestation it was demonstrated that LMWH, when added to low-dose aspirin, is beneficial in preventing recurrent early-onset HD in.[30] Moreover, a beneficial effect of LMWH was seen in women with adverse obstetric history, without thrombophilia.[41] In women with APS, two trials investigated the effect of LMWH on second and third trimester pregnancy complications: the TIPPS trial (n=22) and the FRUIT-RCT (n=32).[42;43] In both studies, no beneficial effect of LMWH was found.
Women with SLE get often low-dose aspirin during pregnancy without the evidence of a trial specifically addressing the effect in SLE pregnancies.[44] The role of LMWH in women with SLE is not established yet. Some studies suggest that women with SLE and APS should be treated with LMWH combined with aspirin.[28;44]

Table 1: current advises in treatment of high risk pregnancies from guidelines of three countries

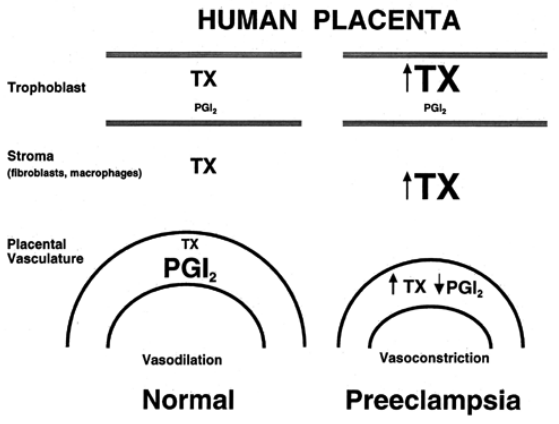
	The Netherlands; NVOG & richtlijnendatabase.nl	Great-Britain; RCOG	America; ACOG	Australia; RANZCOG
Inheritable thrombophilia	Richtlijnendatabase.nl: Prescribe aspirin in women with previous severe HD, irrespective of thrombophilia or APS. Do not prescribe LMWH in women with inheritable thrombophilia, unless in a RCT.	No specific guideline available	Without thrombotic event; Surveillance without anticoagulation therapy With thrombotic event; LMWH or heparin (2013)	No specific guideline available
Antiphospholipid syndrome	Richtlijnendatabase.nl: Prescribe aspirin in women with previous severe HD, irrespective of thrombophilia or APS. Women with obstetric APS: LMWH and aspirin can be considered. NVOG (2007): Based on recurrent spontaneous abortions; LMWH and aspirin Based on adverse obstetric history; Unknown Based on a single thrombotic event; LMWH, aspirin can be considered Based on recurrent thrombotic events: LMWH and aspirin	No specific guideline available	Based on recurrent spontaneous abortions; Heparin and low-dose aspirin can be considered Based on adverse obstetric history; Not mentioned Based on a thrombotic event; Heparin, benefit of aspirin is unknown (2012)	No specific guideline available
SLE without aPL	NVOG (2007): Consider aspirin	No specific guideline available	No specific guideline available	No specific guideline available
SLE with APS	NVOG (2007): LMWH + aspirin	No specific guideline available	No specific guideline available	No specific guideline available
SLE with aPL	Not mentioned in guidelines	No specific guideline available	No specific guideline available	No specific guideline available

Aspirin

The mechanism of action of aspirin is suggested to consist of various aspects.
First some aspects of the physiology of prostacyclin (PGI2) and thromboxane (TX) in pregnancy is elucidated since both factors play a role during pregnancy and in the development of HD. PGI2 is a potent vasodilator and inhibitor of platelet aggregation and uterine contraction.[45-49] The combined effects of PGI2 prevent maternal hypertension and platelet aggregation and promote an increase in uteroplacental blood flow. TX opposes the actions of PGI2 and is a potent vasoconstrictor and stimulator of platelet aggregation

and uterine contraction.[45-47;49] If unopposed by PGI2, TX can lead to maternal hypertension, increased platelet aggregation and decrease of uteroplacental blood flow. Both PGI2 and TX are increased in uncomplicated pregnancies. In pregnancies complicated by HD the production of TX increases 3 to 7 times more than in uncomplicated pregnancies, and PGI2 increases less (Figure 2). With less PGI2 being produced in preeclampsia, the vasoconstrictor effects of TX might not be opposed efficiently, which could lead to HD.[50;51]
The antiplatelet effect of aspirin results from an irreversible inhibition of cyclooxygenase-1 indirectly decreasing the production of TXA_2 (v).[32] In the placenta, aspirin inhibits TX production without significantly affecting PGI2 production. Therefore, the adverse effects of unopposed TX are reduced or eliminated.
Furthermore, aspirin is thought to have an anti-inflammatory effect and inhibits thrombocyte aggregation which can improve the development and efficacy of the placenta.[29]
Suboptimal aspirin serum levels could be influenced by moment of intake or low adherence for aspirin.[52-55] Adherence for aspirin in pregnant women has not been examined yet but has been studied before in non-pregnant patients concluding that around one-third had poor adherence.[56;57]

Figure 2: Balance of thromboxane (TX) and prostacyclin (PGI2) in pregnancy.[51]



Low-molecular-weight heparin
LMWH contains fractioned fragments of heparin with a lower molecular weight than unfractionated heparin. LMWH inhibits both factor Xa and thrombin, although it inhibits factor Xa more effectively than thrombin.[58] Besides the anticoagulant effect, LMWH has possibly an anti-inflammatory and lipase-potentiating effect, decreasing serum lipids.[59;60]
Compared to heparin, LMWH has superior bioavailability and a non-dose-dependent half-live. Therefore, subcutaneous administration once or twice daily is recommend without the need of monitoring the patient its anti-factor Xa level.
Theories about the mechanism of action of LMWH include early effects that may occur at cellular level, by decreasing trophoblast apoptosis and increasing the production of proteases

involved in the trophoblast invasion of the maternal endometrium.[61;62] In vitro studies have shown an effect of LMWH on angiogenesis in the placental villi and show an influence on the dysregulation of soluble vascular endothelial growth factor.[63;64] Furthermore, an inhibiting effect of heparin on complement activation is reported, which could reduce the risk of pregnancy complications.[65-67]

3. Short-term outcomes

Placental disease

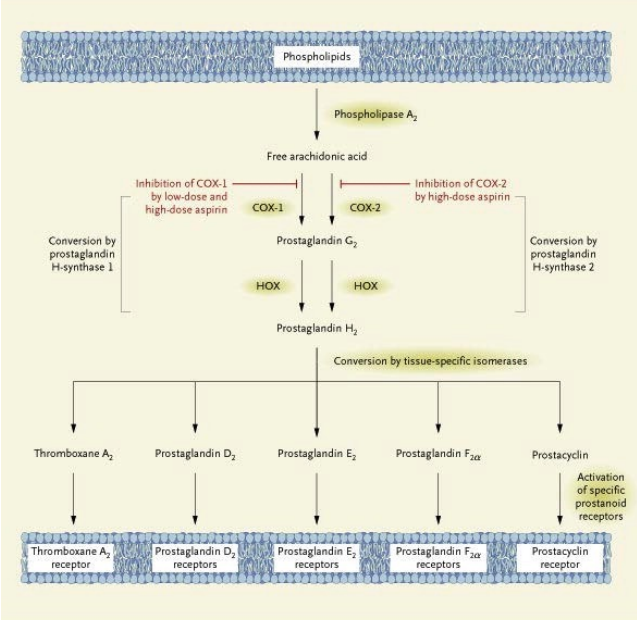
Placental disease is often related to the occurrence of HD and FGR.

Short-term outcomes related to HD are severe maternal morbidity and mortality, as demonstrated in the case in the beginning of this general introduction. Short-term outcomes include maternal organ failure including renal insufficiency, liver involvement and neurological symptoms, varying in severity but sometimes necessitates admission to an intensive care unit. In worst case, the mother dies of the complications related to HD.

Fetal growth reflects the interaction of utero-placental nutrition and the genetically predetermined growth potential of the fetus. FGR has an impact on the neurodevelopment of the fetus and is associated with increased risk of perinatal death and morbidity.[68;69]

Both the pathophysiology of FGR and HD might relate to inadequate conversion of the uterine spiral arteries by the trophoblast, leading to increased placental resistance and secondary to utero-placental insufficiency.[70;71] Doppler ultrasonography can assess both utero-placental and feto-placental blood flow velocities by measuring the flow velocity in the uterine and umbilical artery respectively.[72;73]

Figure 3: mechanism of action of aspirin.[89]



4. Long-term outcomes

Cardiovascular disease

Nowadays, it is commonly accepted theory that pregnancy is a stress test which can identify women who have an elevated risk to develop cardiovascular disease (CVD) later in life.[74-76] Pregnancy can be regarded as a stress test or window for future health.[3] HD can be the first presentation of a disease in the CVD spectrum. It has also been suggested that HD itself is a risk factor for CVD later in life, comparable with other risk factors such as hypertension, hypercholesterolemia and obesity.[77;78] The chance to develop cardiovascular risk factors has been reported to be related to the severity of HD.[74;79] In retrospective studies, it is suggested that women with a history of recurrent HD have a higher chance to develop CVD later in life compared to women with a history of single HD.[80;81]

Alzheimer's disease

Long-term studies examining effects of HD (up to seven years) on cerebrovascular disease, have observed a higher prevalence of subjective cognitive complaints including memory, attention and concentration deficits after several years, compared to women with a history of uncomplicated pregnancies.[82-85] Neuro-imaging studies show the evidence of long-term effects of (pre)eclampsia on the brain, including more frequently and larger cerebral white matter lesions compared to women who have had an uncomplicated pregnancy.[86-88] Both the subjective complaints and the white matter lesions are risk factors for Alzheimer's disease. It is not clear whether there is a relation between HD and Alzheimer's disease later in life.

Outline of this thesis

Projects described in this thesis are a result of a multidisciplinary approach of the departments of obstetrics & gynaecology, internal medicine, rheumatology and Alzheimer Center of the VU University Medical Center and departments of obstetrics & gynaecology and rheumatology of the University Medical Center Utrecht. Moreover, an international collaboration led to an individualized patient data meta-analysis (IPDMA). For this IPDMA we organized the initiating meeting for the protocol with all principal investigators and statisticians per RCT in Amsterdam, kept closed contact during the data mobilization and spend a week in Canada with the principal investigator and statistician of the IPDMA to discuss the analyses and the core message of the article.

The following questions are raised in this thesis:

- Which populations benefit from LMWH and aspirin during pregnancy and what is its effect on mother and fetus/neonate?
- Do adherence for aspirin in pregnant women and aspirin resistance play a role in women with recurrent HD?
- What are the cardiovascular risk factors in women with thrombophilia and a history of single or recurrent (early-onset) HD? Increases a history of HD the risk for Alzheimer's disease?

Anticoagulant and antiplatelet treatment in the prevention of hypertensive disorders of pregnancy:

Low-molecular-weight heparin

Chapter 1 describes the influence of LMWH added to aspirin on flow velocities of the uterine and umbilical artery and on fetal growth. This is examined in women with adverse obstetric history and inheritable thrombophilia, as secondary outcomes of the FRUIT-RCT.
Chapter 2 provides an overview of maternal and fetal outcomes in women with SLE. The SLE flares and obstetric complications are described of all SLE patients in two tertiary centers in the Netherlands over 15 years.
Chapter 3 gives an overview which anti hemostatic treatment strategies are used in women with SLE without aPL, SLE with aPL, SLE with APS and primary APS. Moreover, we investigated maternal and perinatal outcome in relation to anticoagulant and antiplatelet treatment.
Chapter 4 contains the protocol for an individual patient data meta-analysis about LMWH and its use in the prevention of placenta-mediated pregnancy complications.
Chapter 5 presents the results of this individual patient data meta-analysis.

Aspirin

Chapter 6 depicts adherence for aspirin in pregnant women, using two validated questionnaires.
Chapter 7 describes the occurrence of aspirin resistance, using three different tests, in a follow-up study of the FRUIT-RCT. We investigate if aspirin resistance is related to recurrence of HD during the FRUIT-RCT.
Chapter 8 contains the protocol of the RADAR study (Resistance of Aspirin During and After pRegnancy). In this study aspirin resistance will be examined in the three trimesters during pregnancy and postpartum.

Follow-up after hypertensive disorders of pregnancy:

Chapter 9 presents risk factors for cardiovascular disease in women with inheritable thrombophilia and previous single or recurrent HD (follow-up of the FRUIT-RCT).
Chapter 10 examines whether women with Alzheimer's disease had more often HD in history compared to women without Alzheimer's disease, using a validated questionnaire.

Discussion and summary:

The final part contains a general discussion and a summary of this thesis in English and Dutch.

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Does low-molecular-weight heparin influence fetal growth and uterine and umbilical arterial flow-velocities in women with a history of early-onset utero-placental insufficiency and an inheritable thrombophilia? Secondary RCT results.

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Abstract

Objective: Does low-molecular-weight heparin (LMWH) added to low-dose aspirin influence fetal growth and flow-velocity in uterine and umbilical arteries in women with an inheritable thrombophilia and previous early-onset utero-placental insufficiency?

Design: Secondary outcomes of the FRUIT-RCT

Setting: Multi-centre, international

Population: The FRUIT-RCT included 139 women with inheritable thrombophilia before 12 weeks gestation. Inclusion criteria were previous delivery before 34 weeks gestation with a hypertensive disorder of pregnancy and/or small-for-gestational-age infant and an inheritable thrombophilia.

Methods: After randomisation to either daily LMWH with aspirin, or aspirin alone, ultrasound measurements were performed at 22-24, 28-30 and 34-36 weeks gestation. Development over gestation of growth, birth weight and flow-velocity of the umbilical artery was examined using the linear mixed model. Uterine artery flow-velocity at a single time point (22-24 weeks) was examined using a chi-square test.
Main Outcome Measures: Fetal growth over time including birth weight, using Scandinavian, Dutch and customised growth curves, and flow-velocity within the uterine and umbilical arteries.

Results: No difference of fetal growth over time could be demonstrated between the study arms, regardless of which reference criteria were used. The flow-velocity within the uterine artery and umbilical artery did not differ between study arms.

Conclusion: The addition of LMWH to aspirin did not influence either fetal growth and umbilical artery flow-velocity over time nor uterine artery flow-velocity.

Introduction

Low-molecular-weight heparin (LMWH) reduces recurrent early-onset (<34 weeks gestation) hypertensive disorders of pregnancy (HD) when added to aspirin in women with an inheritable thrombophilia.[1,2]

Utero-placental insufficiency can lead to HD and small-for-gestational-age (SGA: birth weight <10th percentile) infants.[3] However, in earlier studies, LMWH did not influence SGA recurrence.[1,2] The exact cause of HD remains unknown, but the leading hypothesis strongly relies on disturbed placentation in early pregnancy.[4]

Fetal growth reflects the interaction of utero-placental nutrition and the genetically predetermined growth potential of the fetus. There is a variety of definitions; the terms FGR and SGA are often used interchangeably.[5,6] A helpful definition of FGR is deflection of the growth curve during pregnancy without defining the extent of the decline. FGR impacts on the neurodevelopment of the fetus and is associated with increased risk of perinatal death and morbidity.[7,8] To analyse growth, different reference growth curves are available. We expected that the recent Dutch growth data[9] and customised growth dataset[10,11] might result in a different neonatal SGA outcome compared with the Scandinavian dataset [12] available at the onset of the FRUIT-RCT[1] because they better reflected our population.

The pathophysiology of HD and SGA infants might relate to inadequate conversion of the uterine spiral arteries by the trophoblast, leading to increased placental resistance and secondary utero-placental insufficiency.[3,13] Doppler ultrasonography can assess both utero-placental and feto-placental blood flow velocities.[14,15] In normal pregnancy, there a rise in diastolic flow-velocity and disappearance of the diastolic notch in the uterine artery waveform.[16] Due to inadequate trophoblast invasion in women with HD, the end-diastolic blood flow-velocity within the uterine artery remains low and the early diastolic notch persists. In the umbilical artery, in normal pregnancy a forward flow-velocity is seen, whereas decreased flow-velocity, with absent or reversed flow-velocity during diastole, is a strong indicator of placental insufficiency.[17]

The aim of the present study was threefold, to examine whether:

1. adding LMWH to maternal treatment with aspirin alters fetal growth over time
2. neonatal SGA is different using the more recent Dutch and customised growth datasets, compared with the Scandinavian dataset used in the FRUIT-RCT.[1]
3. adding LMWH to maternal treatment with aspirin alters flow-velocity within the uterine and umbilical arteries.

These data have provided a unique opportunity of assessing the impact of two different treatments on fetal and neonatal outcomes, with the prospective repeated ultrasound measurements in a very specific population at high risk for FGR.

Methods

This study evaluated secondary endpoints of the FRUIT-RCT.[1] Following informed consent, subjects were randomised to receive either daily low-dose aspirin together with LMWH (Dalteparin, Pfizer Inc., New York, USA, weight adjusted) or daily aspirin alone in women with a previous delivery before 34 weeks with HD and/or a SGA infant, together with an inheritable thrombophilia. LMWH was started between six and twelve weeks gestation, after sonographic confirmation of a viable intrauterine pregnancy and was continued until delivery. Low dose aspirin was also started before 12 weeks gestation and continued until 36 weeks gestation. Specific inclusion and exclusion criteria are discussed in the primary article.1 Between January 2000 and December 2009, 139 women were recruited from seven university hospitals and six non-university hospitals in the Netherlands, two university hospitals in Australia and one university hospital in Sweden.
Ultrasound assessments were performed at 22-24, 28-30 and 34-36 weeks gestation.

Growth over time
During the ultrasound assessments, femur length (FL), abdominal circumference (AC), head circumference (HC) and biparietal diameter (BPD) were measured. These values were used to calculate the Estimated Fetal Weight (EFW) according to the criteria of Hadlock.[18]
There is no generally accepted agreement as to what degree of deflection of the growth curve reflects FGR.19 To measure this deflection and to compare the three EFW values, all EFW measurements were normalised by expressing them as a ratio.[20-25] Using the EFW ratio has several advantages. First, there is no data reduction, because the original measurements are used for the calculations with no dichotomising. Furthermore, deflection in growth over time can be clearly identified because for each measurement the EFW will be normalised allowing for ease of comparison of consecutive EFW ratios.
The EFW ratio uses the median of a reference curve. Outliers have little influence on the median; fetal weight has a normal distribution, with the largest number of observations at the median.
For all three ultrasound measurements, the EFW ratio was calculated using the following formula; EFW ratio = EFW / p50 of Scandinavian, Dutch and customised growth datasets. The 50th centile (p50) from the Scandinavian and Dutch datasets is presented in each of their articles; however, the Dutch dataset only provided data from 25 weeks gestation, so for measurements at 22-24 weeks, an EFW ratio could not be calculated. The p50 of the customised growth data was calculated from the website[26]
In general, an EFW ratio of 1.0 is average; a ratio below 1.0 indicates a fetus smaller than average and a ratio above 1.0 indicates a fetus bigger than average[27] Birth weight was our fourth measurement point, the birth weight ratio was also expressed as the actual birth weight divided by the median value for that gestational age according to each growth dataset.

Neonatal SGA and various growth datasets
In the present study the outcome neonatal SGA has been re-analysed, using Dutch

and customised growth data, and compared with the primary data from the FRUIT-RCT,1 which were analysed using the Scandinavian dataset. While the FRUIT-RCT was in progress, a Dutch dataset was published (based on 176,000 singleton births in the Netherlands in the year 2001). Moreover, weight at each gestational week was customised by individual profile. It was based on the observation that birth weight varies with maternal parameters including ethnicity, parity and pre-pregnancy weight, and with fetal sex.[10;11]

Doppler flow-velocity measurements

During the first assessment at 22-24 weeks gestation, flow-velocity within the uterine artery was measured and the Resistance Index (RI: peak systolic velocity minus end diastolic velocity divided by peak systolic velocity) calculated. Assessment of the uterine artery was not repeated later in the pregnancy, given that uterine artery flow-velocity does not change significantly after the second wave of trophoblast invasion has been completed.

At the same occasion and at the two subsequent time points, flow-velocity within the umbilical artery was measured and the Pulsatility Index (PI: Peak systolic velocity minus end diastolic velocity divided by mean velocity) calculated. As the umbilical arterial PI changes over time during pregnancy, it has been expressed as a ratio. The measured value is divided by the median value for that specific gestational age,[28] using the formula:

PI ratio = measured PI / p50 PI of control population[29]

In general, a PI ratio of 1.0 is average; a ratio greater than 1.0 indicates a higher resistance than average with reduced flow-velocity.

Definitions

HD: pre-eclampsia, haemolysis, elevated liver enzymes and low platelets (HELLP) syndrome and eclampsia SGA: birth weight less than 10th percentile using various datasets. Decreased flow-velocity within the uterine artery: mean RI of the left and right uterine arteries > 0.60 and/or presence of a notch within the waveform.

Statistics

Raw data of fetal growth per ultrasound measurement and birth weight between study arms were examined using an independent t-test.

To analyse differences in fetal growth over time and in flow-velocity of the umbilical artery between study arms, a linear mixed model was used, using the EFW ratio (including birth weight ratio) and PI ratio respectively. The linear mixed model facilitates comparison of two groups and corrects for several missing values at different moments and for different start and endpoints.[30]

For fetal growth over time, linear mixed model analyses were performed using all three datasets. To evaluate if the birth weight ratio could be used in the linear mixed model, a Pearson correlation test was performed to examine whether the birth weight ratio correlated with the last EFW ratio. We performed additional analyses with the linear mixed model to investigate if there was any effect modification for smoking, HD in index pregnancy, SGA in index pregnancy, and maternal BMI above 25 kg/m^2 before pregnancy. This was done for

EFW ratio and birth weight ratio using all three datasets.
To compare neonatal SGA for the Dutch and customised growth datasets between arms, as well as to compare abnormal flow velocities within the uterine arteries between study arms, a chi-square test was used.
Statistical analyses were performed with IBM SPSS 20.0 (SPSS Inc, Chicago, IL). Results were considered significant at the 5% level.

Results

Baseline characteristics are shown in Table 1, with the additional Dutch and customised growth data in the assessment of SGA. The previous report described outcome data from 139 women who participated in the FRUIT-RCT, with one twin pregnancy, five miscarriages and one intra-uterine fetal death at 17 weeks gestation not caused by HD [1] The number of data points per ultrasound measurement available for statistical analysis are depicted in Table 2. Reasons for missing data included premature birth, no reference data available for the Dutch growth parameters before 25 weeks of gestation, and cancelled appointments. To calculate customised growth, information was needed, including maternal ethnicity, parity and pre-pregnancy weight, and fetal sex. Missing one of these parameters explains the lower number of complete data points, compared with Scandinavian and Dutch data. The majority of women were of Dutch origin. Fourteen women were non-Caucasian.

Fetal Growth over time
Mean EFW per ultrasound measurement and mean birth weight are given in Table 3. The last measured EFW ratio correlated with the birth weight ratio (correlation 0.590, P<0.001, data not shown), therefore birth weight was used as a fourth measurement in the growth curve. The change over time of the EFW ratio and the birth weight ratio showed no difference between study arms, whichever reference dataset was used (Scandinavian P=0.68, Dutch P=0.28, or customized P=0.22). No effect modification was found for smoking, HD in index pregnancy, SGA in index pregnancy, and maternal BMI >25 kg/m² before pregnancy (data not shown).

Neonatal SGA and various growth datasets
As in the previously reported data from the FRUIT-RCT1, which were analysed using the Scandinavian dataset, the present study has also shown no difference in neonatal SGA according to the Dutch (25% SGA, P=0.76) and customised growth datasets (34% SGA, P=0.37). Of the six women with early-onset HD in the original trial, all in the aspirin only arm, four had a SGA infant according to the Scandinavian dataset and five an SGA infant according to the customised growth dataset. However, the Dutch dataset only starts at 25 weeks gestation. Three of the six women with early-onset HD in the trial delivered before 25 weeks gestation and so SGA could only be assessed according to the Dutch dataset in the other three, two of whom were SGA according to these criteria.

Table 1: Baseline characteristics of the FRUIT-RCT1 population, adapted with data for this study

	LMWH with aspirin (N=70)	Aspirin alone (N=69)
Index pregnancy		
Maternal age – years	29.1 ± 4.7	29.2 ± 4.4
Non-Caucasian	4 (5.9)	10 (14.9)
Diagnosis in index pregnancy		
Hypertensive disorder of pregnancy	58 (82.9)	49 (71.0)
Pre-eclampsia*	48 (68.6)	40 (58.0)
HELLP syndrome*	33 (47.1)	28 (40.6)
Eclampsia*	2 (2.9)	3 (4.3)
Small-for-gestational-age*	49 (70.0)	45 (65.2)
Scored according to Scandinavian data[12]	48 (71.6)	47 (67.1)
Scored according to Dutch data[9]	21 (30)	19 (27.1)
Scored according to customised growth data[10,11]	53 (75.7)	53 (75.7)
Results		
Gestational age at delivery – days	208.2 ± 21.5	205.7 ± 20.8
Gestational age at delivery 16-24 weeks	3	3
Birth weight – grams	1020.5 ± 422.3	978.6 ± 486.9
Fetal death / neonatal death <28 days	23 (32.9)	26 (37.7)
Thrombophilia disorder		
Protein C deficiency*	3 (4.3)	4 (5.8)
Protein S deficiency*	12 (17.1)	12 (17.4)
Activated Protein C resistance (APCr)	2 (2.9)	2 (2.9)
APCr + Factor V Leiden mutation*	38 (54.3)	44 (63.8)
Prothrombin gene G20210A mutation	23 (32.9)	8 (11.6)
Diverse: Factor XII deficiency	0	1
Hyperhomocysteinaemia	8 (15.1)	9 (16.7)
Pregnancy data at entry		
Maternal age – years	31.9 ± 4.6	31.6 ± 4.7
Maternal age 36 years or older	15 (21.4)	14 (20.3)
Gestational age – days	64.8 ± 15.0	63.4 ± 12.8
Parity	1.1 ± 0.3	1.3 ± 0.6
Gravidity	2.5 ± 0.9	2.6 ± 1.0
Body mass index (BMI) – kg/m^2	26.8 ± 6.0	26.3 ± 5.7
BMI ≥25 kg/m^2	33 ± 51.6	28 ± 45.2
Chronic hypertension	16 (22.9)	12 (17.4)
Antihypertensive drug treatment	13 (18.6)	11 (15.9)
Daily smoking	4 (6.7)	9 (14.8)
Family history of vascular disease*:	33 (52.4)	22 (34.9)
1st degree family: arterial	22 (34.9)	15 (23.8)
1st degree family: venous	8 (12.9)	7 (11.1)
mother and/or sister: pre-eclampsia	9 (14.3)	3 (4.8)

Values represented as means ± SD or absolute numbers (%) as appropriate. *Some women had more than one diagnosis during index pregnancy, thrombophilia disorder or aspect of vascular disease in the family.

Flow-velocity within the uterine and umbilical arteries

The mean uterine artery RI was not different in the two study arms (Figure 1). Decreased flow-velocity within the uterine artery was found in 44/92 women (47.8%): 20/43 (46.5%)

in the LMWH + aspirin arm, and 24/49 (49.0%) in the aspirin only arm (p= 0.81)
The ratio of the flow-velocity within the umbilical artery was not different in the two study arms with no difference in flow-velocity over time (P=0.19) (a and 2b).

Table 2: Numbers of data points available for statistics per ultrasound measurement.

	22-24 weeks		**28-30 weeks**		**34-36 weeks**	
	LMWH + aspirin	**Aspirin only**	**LMWH + aspirin**	**Aspirin only**	**LMWH + aspirin**	**Aspirin only**
Fetuses	66	67	66	63	63	61
Performed EFW measurements						
Total	59 (89%)	59 (88%)	60 (91%)	57 (90%)	51 (81%)	52 (85%)
Available EFW ratios						
Scandinavian data	58 (88%)	57 (85%)	60 (91%)	57 (90%)	51 (81%)	51 (84%)
Dutch data	n.a.	n.a.	60 (91%)	57 (90%)	51 (81%)	51 (84%)
Customised growth data	53 (80%)	52 (78%)	51 (77%)	48 (76%)	44 (70%)	42 (69%)
Performed flow-velocity measurements						
RI uterine artery*	43/66 (65%)	49/66 (74%)	NM	NM	NM	NM
PI umbilical artery	42 (64%)	45 (67%)	50 (76%)	48 (76%)	49 (78%)	47 (77%)

Weeks is a synonym for weeks of gestational age. LMWH: Low-molecular-weight heparin; EFW: Estimated Fetal Weight;n.a: data not available (Visser9 provided data from 25 weeks gestational age); RI: Resistance Index; NM: Not measured (see methods); PI: Pulsatility Index.
* number of women not identical to number fetuses because of one twin pregnancy Performed EFW measurements; total number of performed ultrasounds measuring the EFW.
Available EFW ratios; to calculate the EFW ratio, also the p50 per reference must be available. In some cases, the EFW measurement was performed, but reference data were not available. Performed flow-velocity measurements; total number of performed ultrasounds measuring the RI of the uterine artery and PI of umbilical artery.
Values are represented as absolute numbers (percentage of total ultrasounds that could have been performed).

Discussion

Main findings

his study in a very select high risk population of pregnant women has shown no difference over time in fetal growth (including birth weight) or in flow-velocity of the umbilical artery, and no difference in the flow-velocity within the uterine artery at a single time point, between women who used LMWH combined with aspirin, or women who used aspirin only.
Nor was there a difference in neonatal SGA between the study arms, independent of which reference dataset was used, which is in line with Gris et al.[31]
The high risk of the examined population for utero-placental insufficiency was confirmed, 25%-34% having a neonatal SGA. It was expected that women participating in the trial would have had a high risk for recurrent HD (about 20%). Since the primary outcome of the trial, early-onset HD, did differ between the arms, we were especially interested in the six outcomes before 34 weeks. The proportion of neonatal SGA in these six infants was high, dependent on which reference range was used: 66-83%.
Although there were no outcome differences between the study arms that could be

Table 3: Mean EFW and birth weight per study arm

Period	LMWH+ aspirin	Aspirin only	*P*
22-24 weeks	n=59	n=59	
	557.5 (182.4)	561.7 (162.7)	0.89
28-30 weeks	n=60	n=57	
	1394.6 (294.5)	1401.8 (215.2)	0.88
34-36 weeks	n=51	n=52	
	2366.8 (349.7)	2446.2 (399.1)	0.29
Birth	n=66	n=68	
	3061.7 (740.7)	2899.2 (965.9)	0.28

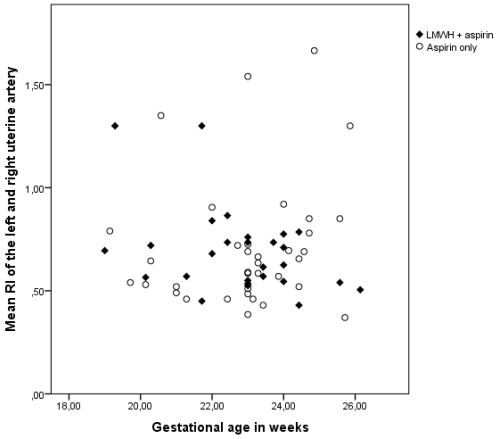
Values represented as means (SD) in grams.
P is calculated using an independent t-test.
EFW: estimated fetal weight. LMWH: Low-molecular-weight heparin. Weeks is a synonym for weeks of gestational age.

ascribed to treatment, we consider it remarkable that the flow-velocity within the uterine artery was decreased in 47.8% of the study pregnancies, reflecting a high risk of suboptimal placentation in these women.
Although there were no differences in growth and umbilical artery flow-velocity over time and in uterine artery flow-velocity at a single time point in mid-pregnancy after the completion of the second wave of placentation, there remains an indication to add LMWH to aspirin in this specific population because of the reduction in recurrent early-onset HD[1,2]

Strengths and limitations
The FRUIT-RCT is one of the largest clinical trials performed in women with a specific obstetric history (HD or a SGA infant and delivery before 34 weeks) and an inheritable thrombophilia. The strengths of this study of the secondary outcomes from the trial are the prospective growth and Doppler ultrasound measurements, the strict inclusion criteria and the early start of treatment, the meta-analysis of Bujold et al[32] having concluded that commencing aspirin early in pregnancy (< 16 weeks gestation) reduces the risk of HD and FGR. Furthermore, we used for our analysis the linear mixed model for the EFW ratio including the birth weight ratio and for the PI ratio of the umbilical artery. This provides a very accurate evaluation, in contrast to dichotomised values[19] This does not mean that working with a ratio should replace dichotomisation, but it is an important addition to the current methods especially when calculating FGR and SGA. The weakness of this study is the missing data; generally due to the fact that some women had had an early delivery or had cancelled appointments and so may not be missing at random. However, there is a discrepancy between the missing fetal growth and flow-velocity data, so there might be a response bias. In addition, though large in comparison with previous studies, the number of studied women is small, which may have made finding differences difficult.

Interpretation
Our results in relation to fetal growth over time are in line with the data from the HAPPY trial,[33] which similarly did not find an effect of LMWH on FGR, although the authors used

Figure 1: Mean RI of the left and right uterine arteries in the two study arms.
RI: Resistance Index.



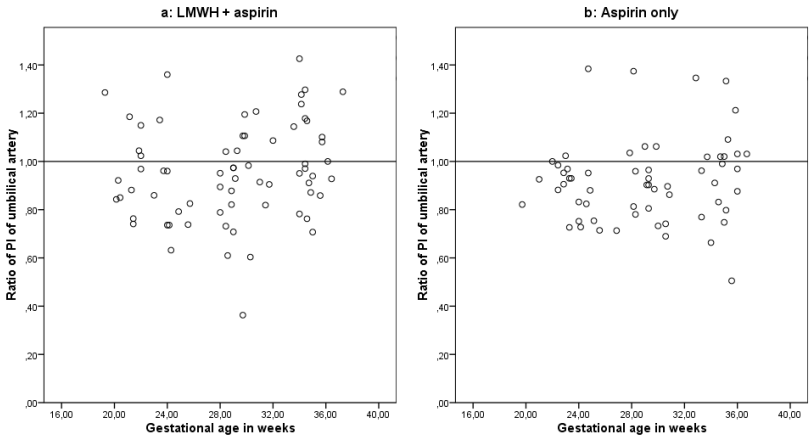
a different definition of FGR: birth weight below the 10th percentile, including a percentile reduction of the abdominal circumference of more than 40%. Compared with our study, the HAPPY trial recruited women with a wider range of previous obstetrical complications.[33] In one study in pregnant women with inheritable thrombophilia that showed a positive effect of LMWH on FGR, a decrease of 30% in FGR was seen when LMWH was combined with aspirin compared with treatment with LMWH and aspirin separately.[34] This, however, was a retrospective cohort study with wider inclusion criteria and several pregnancies per woman were included

Using the various growth datasets showed no different effects on the outcome. Most women in this study were of Dutch origin. Currently, Dutch women and their children are among the tallest populations in the world.[35] The Dutch dataset could potentially have been the most useful reference, but it did not prove to be so.

A study performed in a mixed population in the USA showed that, when SGA was defined using a customised growth curve, substantially more pregnancies were at risk for adverse outcomes when compared with those defined using standard growth curves.[36] This was further supported by a Swedish study and a French study. [37;38] However, an earlier American study concluded that neither the use of ultrasound-based nor the individualised growth reference charts did well in predicting adverse perinatal outcomes.[39] The authors suggested that their result could probably be explained because their study population was relatively homogeneous: a predominantly white population, and thus comparable with our trial. So, although of importance in a more mixed population, the ethnicity-related database of Gardosi[10;11] was not of additional value in the present study.

The severity of the obstetrical history may influence the effect of LMWH on uterine and umbilical flow-velocity. In another population with wider inclusion criteria i.e. SGA, severe early-onset HD, recurrent miscarriages and intra-uterine fetal death later than 16 weeks gestation, an improvement in the PI of the uterine artery was demonstrated and no effect was seen in the flow-velocity within the umbilical artery after treatment with either aspirin

Figure 2a+b: Ratio of the flow-velocity in the umbilical artery in the two study arms. PI: Pulsatility Index. The reference line represents the average ratio of the PI of the umbilical artery in a control population.[29]



or both aspirin and LMWH.[40] However, this was a non-randomised study including women with either inheritable or acquired thrombophilia, whereas we have only investigated women with inheritable thrombophilia in the context of an RCT.

Conclusion

This study has focused on fetal growth over time including birth weight, and on flow velocities of the uterine and umbilical arteries, in a population with a previous delivery before 34 week of gestation associated with HD and/or a SGA infant and an inheritable thrombophilia. Our conclusion is that treatment with LMWH influences neither fetal growth, independent of which reference dataset is used, nor flow velocities of the uterine and umbilical arteries. There is no current consensus on whether the addition of LMWH to aspirin in women with thrombophilia and an adverse obstetric history has an influence on fetal growth, so further research is needed. Homogeneity in this population resulted in the finding that no differences were found in fetal growth whether the Scandinavian, Dutch or customised growth datasets were applied. This specific population has an impressively high risk both for neonatal SGA (~30%) and for decreased flow-velocity within the uterine artery (~48%). Despite the fact that we did not find differences in growth and umbilical flow-velocity over time and in uterine flow-velocity at a single time point mid-pregnancy, there remains an indication to add LMWH to aspirin in this specific population since the combination treatment reduces recurrent early-onset HD.[1,2]

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appendix 1

Letter-to-the editor and author's reply on a letter in which elucidation of two aspects of the article was requested. Authors reply

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Carolien N.H. Abheiden
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Sir,

We thank Drs. Griffin and King for their interest in our article[1] They invited us to elucidate two aspects. First, they were concerned at the absence of a physiological explanation for treatment with low-molecular-weight heparin (LMWH) to prevent birth of small-for-gestational age (SGA) infants. While we agree that this aspect was limited in our paper, we had earlier described plausible reasons for an effect of LMWH when added to aspirin in the prevention of hypertensive disorders of pregnancy (HD) and SGA, with their frequent association with utero-placental thrombosis, in the primary publication of the FRUIT-RCT.[2] In this, we remained modest about the exact pathway of such an effect. We surmised, however, that the pathway is not limited to an anticlotting mechanism and commented that both an effect on angiogenesis in the placental villi and an influence on the dysregulation of soluble vascular endothelial growth factor have been seen with LMWH. Moreover, we pointed out that such an influence is likely to occur during the first part of the second wave of trophoblast invasion, given the marked reduction in early-onset HD (ie, prior to 34 weeks of gestation) seen in our trial.

Your correspondents also commented on a possible discrepancy between the final paragraph of the present study1 and the conclusions of a meta-analysis on recurrent placenta-mediated pregnancy complications.[3] We appreciate the opportunity to explain the misunderstanding. The present study has reported data from the FRUIT-RCT, which had as its primary outcomes both recurrent early-onset HD and HD irrespective of gestational age: early-onset recurrence of HD was reduced using LMWH and aspirin, whereas recurrence of HD irrespective of gestational age was not.[2] The primary outcome of the meta-analysis was a composite of preeclampsia (irrespective of gestational age), placental abruption, SGA <10th centile and pregnancy loss >20 weeks.[3] The authors found that LMWH reduced this composite outcome although they did not find this effect in the two highest quality trials (including the FRUIT-RCT). The discrepancy can be explained easily by the fact that these trials were compared for the outcome parameter "preeclampsia irrespective of gestational age" and not for early-onset HD. Thus, the metanalysis underlines our FRUIT-RCT results, ie no reduction of recurrent HD irrespective of gestational age, and does not contradict them. We are keen to explore the different outcome for early onset HD, and so we are currently contributing to an individual patient data meta-analysis of the same trials of the meta-analysis.[3;4]

In summary, we conclude that, despite not finding differences either in fetal growth restriction over time or in uterine and umbilical flow velocities in our trial participants, the reduction of recurrent early onset HD seen in our trial supports the use of combination LMWH and aspirin therapy in the specific population of women with prior early-onset HD or SGA, birth before 34 weeks and inheritable thrombophilia.

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appendix 2

Letter-to-the editor and author's reply on a letter entitled 'anticoagulant therapy and cell-derived microparticles' Author's reply

BJOG. 2016 Apr;123(5):844-5

Carolien N.H. Abheiden
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Sir,

We thank Drs Patil, Ghosh and Shetty for their careful reading of our article and their interest in the subject.[1] They suggest that cell-derived procoagulant microparticles (MPs) might help to explain the positive effect of low-molecular-weight heparin (LMWH) in the prevention of hypertensive disorders of pregnancy (HD) as well as its lack of impact on fetal growth restriction (FGR). We are aware of these possible role of MPs, also described in other studies.[2] The authors suggest that trials investigating the effect of LMWH on pregnancy outcome should also analyse for MPs concentration.

We agree that the role of MPs in the pathophysiology of HD and FGR should be further elucidated. The FRUIT-RCT started in 2000, and was designed in the late nineties.[3] At that time, little was known about the role of MPs in pregnancy. To our knowledge, the first article about MPs and pre-eclampsia was published in 2002,[4] and so analysis of MPs was not included in the design of the trial. A further option for future studies would be to investigate different phenotypes of the MPs and their role in the development of HD and FGR.

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2

Maternal and perinatal outcome in women with systemic lupus erythematosus, a retrospective multicenter cohort-study

Submitted

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Abstract

Objective: To investigate disease activity around and during pregnancy and pregnancy outcome in women with systemic lupus erythematosus (SLE) taking antiphospholipid antibody status into account. Moreover, differences between first and consecutive pregnancies and number of live births were examined.

Methods: All ongoing pregnancies (>16 weeks gestation) of SLE patients receiving joint care from rheumatologists and gynecologists in two tertiary centers in the Netherlands between 2000-2015 were included. Disease activity (SELENA-SLE(P)DAI around and during pregnancy), flare rate, pregnancy complications and number of live births were assessed by medical chart review.

Results: From 96 women (84% Caucasian) 144 pregnancies were included. The median SELENA-SLE(P)DAI score was 2 before (<6 months), during and after pregnancy (<6 months) and flare rates were 6.3%, 20.1% and 15.3% respectively. Severe hypertensive disorder of pregnancy (preeclampsia, eclampsia or HELLP-syndrome), intrauterine fetal death, preterm birth and small-for-gestational age infants occurred in 18.1%, 4.1%, 32.7% and 14.8% respectively. Only HELLP-syndrome occurred more often in women with SLE and antiphospholipid syndrome compared to SLE women with or without antiphospholipid antibodies. Pregnancy complication rates were similar in first and consecutive pregnancies. Half of the women did not experience any pregnancy complication during their studied reproductive period, whereas 42.7% developed a complication during all pregnancies. Mean number of pregnancies was 2.4 and live births 1.7.

Conclusion: In a multidisciplinary monitored SLE population with low disease activity, maternal and perinatal complications were nearly equally distributed, irrespective of antiphospholipid antibody status or first and consecutive pregnancies. This information is useful for patient counseling.

Introduction

Systemic lupus erythematosus (SLE) is a systemic auto-immune disease that often affects women during their childbearing age.[1] It is well known, that women with SLE may experience an increase in disease activity during pregnancy.[2-4] Moreover, women with SLE have a higher risk of experiencing pregnancy complications like hypertensive disorders of pregnancy (HD: pregnancy induced hypertension (PIH), preeclampsia, eclampsia and hemolysis, elevated liver enzymes, low platelets (HELLP)-syndrome, preterm birth, intrauterine fetal death (IUFD) and small-for-gestational age (SGA) infants compared to the general population.[4-7] Several risk factors for pregnancy complications in women with SLE have been reported. Among them are the presence of antiphospholipid antibodies (aPL) or antiphospholipid syndrome (APS), (prior) lupus nephritis and active disease at conception.[8-10] Therefore, low disease activity for at least six months is recommended to lower the risk for SLE flares and maternal and perinatal complications.[11-14] In order to achieve this, preconceptional counseling and close collaboration between gynecologist and rheumatologist are recommended.[10,11,15] Evaluation of risk factors (e.g. smoking, hypertension, overweight, family history) and optimization of timing of pregnancy are goals of preconceptional counseling. Moreover, the use of pregnancy compatible medication, amongst others azathioprine and hydroxychloroquine (HCQ), is evaluated in order to prevent flares and maternal and perinatal complications.[16]

Over the last decades, an improvement in pregnancy outcomes in SLE patients has been reported.[17] A recent large North-American multicenter study investigated one pregnancy per woman with SLE (n=385), excluding patients with comorbidity such as diabetes or impaired renal function and patients using medium or high dose glucocorticosteroids. The results of this study demonstrated that 80% of the neonates was born alive after a gestation period >36 weeks, not including miscarriages.[18] In the present study, pregnancies of women with SLE over a 16 year period, irrespective of comorbidity and medication use, are described.

In the general population, HD and PIH occur most commonly in first pregnancies.[19] This has not been examined yet in a population with SLE, where several factors (e.g. underlying immune activation, impaired renal function or APS) might be associated with a higher incidence of HD and other pregnancy complications also in consecutive pregnancies.

The aim of the present study is to examine three topics, taking the antiphospholipid antibody status into account:

1. SLE disease activity before, during and after pregnancy per pregnancy;
2. Maternal and perinatal complications occurring in first and consecutive pregnancies and during the reproductive period;
3. Total number of live births per patient.

The results of this study will provide relevant information for health care professionals who are involved in the treatment and preconceptional counseling of these patients and their partners.

Patients and Methods

This cohort study involved two tertiary centers in the Netherlands: the University Medical Center Utrecht and the VU University Medical Center in Amsterdam. To identify pregnancies in women with SLE, a search was performed in both obstetric and rheumatology databases. Data were retrieved from medical files and collected in both centers using the same case report form. The Institutional Review Boards of both university hospitals concluded that official approval from a medical ethical committee was not needed due to the observational character of this study.

Participants

Inclusion criterion was diagnosis of SLE according to the American College of Rheumatology (ACR) revised criteria,[20] diagnosed before start of the first recorded study pregnancy. Moreover, only patients with both obstetric and rheumatology check-ups during pregnancy in one of the two participating centers were included. All ongoing pregnancies (>16 weeks of gestation) between the years 2000 and 2015 were included. No exclusion criteria were formulated.

Antiphospholipid antibody status was recorded in all patients. Patients were divided into SLE without aPL, SLE with aPL or SLE with APS. Presence of aPL was defined as two positive measurements of either IgG or IgM anticardiolipin antibodies or lupus anticoagulant, measured at least six weeks (before 2006) or 12 weeks (after 2006) apart and, when applicable, not during pregnancy or within ten weeks thereafter.[21;22] In 29.9% of the pregnancies presence of beta2-glycoprotein antibodies was measured as well. Samples were considered positive for anticardiolipin antibodies or beta-2-glycoprotein antibodies when either above 40GPL, 40MPL or above the 99th percentile. APS was diagnosed according to the Sapporo criteria.[21]

Outcomes

Baseline characteristics included information about aPL status, demographic background, age, body mass index (BMI), general and obstetric history. The obstetric history included miscarriages (<16 weeks gestation), severe HD (preeclampsia, eclampsia and HELLP-syndrome), IUFD preterm birth (<37 weeks) and SGA infants (birth weight <p10).

Disease activity was assessed in retrospect using the Safety of Estrogens in Lupus Erythematosus National Assessment (SELENA) Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) within 6 months before pregnancy and within 6 months postpartum.[23] In each trimester of pregnancy disease activity was assessed using SLEPDAI (SLEDAI adjusted for pregnancy).[24] Flares were defined according to the SELENA SLEDAI definitions.[23] Clinical manifestations of SLE (according to the revised ACR criteria[20]) and (changes in) dosages of medication were registered. Anticoagulation therapy during pregnancy of this population has been published.[25]

The following maternal and perinatal complications were scored: mild HD (PIH), severe HD, IUFD, preterm birth (both <36 and <37 weeks gestation), SGA infants and occurrence of

neonatal lupus: either cutaneous lupus or congenital heart block. Occurrence of HD (and thereby distinction with nephritis) was scored by one of the gynecologist (ATL and JIPdV).
In the first analysis, maternal and perinatal complications were described for all pregnancies meeting the inclusion criteria; secondly a comparison between first and consecutive pregnancies meeting the inclusion criteria was undertaken. In the third analysis all complications per included patient during the studied reproductive period were examined. The latter was defined as the study pregnancies during the 16-year period and prior obstetric history.
Data are presented per total SLE population and per any of three subdivisions: aPL absent, aPL present but not fulfilling APS criteria and aPL present plus fulfillment of APS criteria.
Total number of pregnancies, total number of live births and miscarriage rate per patient were examined during the studied reproductive period.

Statistics
Baseline characteristics were examined per antiphospholipid group using Fisher's exact test or Chi-square test for dichotomous variables and independent samples median test or one-way ANOVA for continuous variables.
Differences in disease activity and start or increase of prednisone, azathioprine and HCQ dose between the three antiphospholipid groups were tested using independent samples median test for continuous variables or Fisher's exact test for dichotomous variables. Total number of flares during pregnancy compared with the total number of flares postpartum was examined using a Fisher's Exact test.
Differences in incidence rates of maternal and perinatal complications between the three aPL subdivisions were investigated using generalized estimating equations, for which an exchangeable correlation structure was chosen. This analysis corrects for patient dependency since some women in our cohort were included with multiple pregnancies. All outcomes were corrected for smoking, body mass index (BMI) >25 kg/m^2 and prednisone use.
Differences in maternal and perinatal complications rates between first and consecutive pregnancies were examined using a Chi-square test or Fisher's exact test.
Maternal and perinatal complications rates and numbers of live births in the studied reproductive period were studied using descriptive statistics.
Statistical analysis was performed using SPSS for Windows (version 22, SPSS Inc., Chicago, IL, USA). A two-sided *p*-value inferior to 0.05 was considered to be statistically significant.

Results

In total 96 patients with 144 pregnancies met the inclusion criteria. Distribution of the parity was 70 nulliparous women, 18 primiparous women, 7 women were para 2 and 1 woman was para 4 at the first included study pregnancy. Baseline characteristics are presented in Table 1. In the group of 10 patients with SLE and APS, nine had a history of thrombotic APS and four obstetric APS. LAC status was positive in 28.6% of the patients with SLE and aPL and positive in 84.6% of the patient with SLE and APS. Of the non-Caucasian patients,

Table 1: Baseline characteristics per study pregnancy.

	Total	SLE - aPL	SLE + aPL	SLE + APS	p-value
Pregnancies (n)	144	117	14	13	
Number of women	96	77	9	10	NA
Study pregnancies per woman	1 [1-2]	1 [1-2]	1 [1-2]	1 [1-1.5]	0.71
Non-Caucasian#	15/91 (16.5)	13/74 (17.6)	2/8 (25.0)	0/9 (0)	0.36
Age (years)	31.9 ± 4.4	32.1 ± 4.4	29.7 ± 4.0	32.5 ± 4.7	0.16
BMI (kg/m^2)	23.7 ± 4.4	23.2 ± 3.5	25.2 ± 3.6	25.9 ± 9.0	0.05
Smoking	12/139 (8.6)	10/113 (8.8)	0/14 (0)	2/12 (16.7)	0.28
General history					
Chronic hypertension	20/142 (14.1)	16/117 (13.7)	2/13 (15.4)	2/12 (16.7)	0.81
Diabetes	5/143 (3.5)	4/117 (3.4)	0/14 (0)	1/12 (8.3)	0.42
History of thrombosis*	23/144 (16.0)	14/117 (12.0)	0/0 (0)	9/13 (69.2)	<0.01
Serum creatinine level <6 months before pregnancy (µmol/L)	67.2 ± 11.4	67.6 ± 10.9	69.0 ± 13.4	62.6 ± 13.2	0.31
SLE specific history					
SLE duration before start pregnancy (years)	7.8 ± 4.9	7.7 ± 5.0	8.7 ± 4.2	7.6 ± 4.6	0.76
History of nephritis^	57/144 (39.6)	45/117 (38.5)	7/14 (50.0)	5/13 (38.5)	0.70
SS-A and/or SS-B positive	70/138 (50.7)	31/111 (55.0)	6/14 (42.9)	3/13 (23.1)	0.08
Medication use at start pregnancy					
Hydroxychloroquine	69/135 (51.1)	54/109 (49.5)	6/13 (46.2)	9/13 (69.2)	0.38
Azathioprine	39/140 (27.6)	35/114 (30.7)	3/13 (23.1)	1/13 (7.7)	0.21
Prednisone	74/140 (52.9)	63/114 (55.3)	7/13 (53.8)	4/13 (30.8)	0.25
Obstetric history					
Miscarriages**	32/94 (34.0)	26/78 (33.3)	2/8 (25.0)	4/8 (50.0)	0.68
Severe HD	19/63 (30.2)	16/51 (31.4)	2/7 (28.6)	1/5 (20.0)	1.00
IUFD	13/91 (14.3)	13/76 (17.1)	0/8 (0)	0/7 (0)	0.33
Preterm birth (<37 weeks)	24/76 (31.6)	21/64 (32.8)	2/7 (28.6)	1/5 (20.0)	1.00
SGA infant	17/69 (24.6)	16/57 (28.1)	0/14 (0)	1/5 (20.0)	0.34

Data depicted as numbers (%), mean ± standard deviation, or median [interquartile range].
This item is depicted per woman, not per pregnancy, ^biopsy proven, *either arterial or venous, **16 weeks gestation.
SLE: systemic lupus erythematosus, aPL: antiphospholipid antibodies, APS: antiphospholipid syndrome, NA: not applicable, BMI: body-mass index, Severe HD: hypertensive disorders of pregnancy including preeclampsia, eclampsia and HELLP-syndrome, IUFD: intrauterine fetal death, SGA: small-for-gestational age (birth weight <p10).

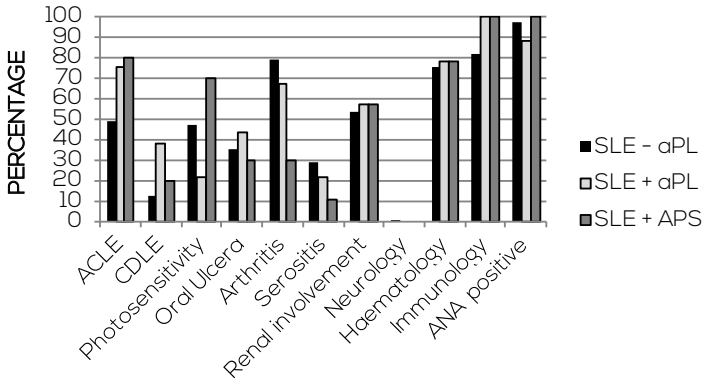
eight were black and seven women were Asian. Thirty-three percent of all patients had a BMI above 25 (kg/m^2). None of the patients had a platelet count below 100*10^9/L at start of the pregnancy.

SLE manifestations and disease activity before, during and after pregnancy

The percentages of the different ACR criteria which were present at the start of the first included study pregnancy are depicted per aPL subdivision in Figure 1. There were no differences in prevalence of the ACR criteria between both centers (data not shown).

Results of disease activity measurements according to the SELENA SLE(P)DAI criteria before and during pregnancy and postpartum are presented in Table 2. During pregnancy, 20.1% (n=29) of the patients developed a flare. Of nine pregnancies in which a mild flare occurred within six months before pregnancy, five experienced consecutive flares either during pregnancy (n=1), postpartum (n=3) or both (n=1). Severe flares occurred three times during pregnancy and twice postpartum. These severe flares were characterized by (amongst

Figure 1: Percentage of women fulfilling each ACR criterion at start of the first recorded pregnancy.



ACR: American College of Rheumatology, SLE: systemic lupus erythematosus, aPL: antiphospholipid antibodies, APS: antiphospholipid syndrome, ACLE: acute cutaneous lupus erythematosus, CDLE: chronic discoid lupus erythematosus, ANA: antinuclear antibodies.

others) nephritis, pleuritis and rash. One patient had both mild flares (during pregnancy) as well as a severe flare (postpartum).

Flare rates postpartum were lower than during pregnancy (15.3 vs 20.1% respectively, p<0.01). Out of 22 pregnancies in which a flare occurred postpartum, [14] had also a flare before or during pregnancy (63.6%, p<0.01). In total, 61 flares occurred in 44 pregnancies.

Treatment with prednisone, azathioprine or HCQ was started or dosages were increased during pregnancy in 17%, 4% and 3% of the pregnancies, respectively. Frequencies of initiation or dose increase of prednisone or azathioprine during pregnancy did not differ per antiphospholipid group (p=0.77 and p=0.72, respectively). Initiation or dose increase of HCQ during pregnancy was more frequent in patients with SLE + APS compared to the other two groups (p<0.01). In 54 pregnancies HCQ was used. Comparing treatment before and after 2008, the use of HCQ during pregnancy increased: 16% received HCQ before 2008 and 58% after 2008 (p<0.01). Flare rate during pregnancy (p=0.09), occurrence of severe HD (p=0.31), IUFD (p=0.20) or preterm birth <37 weeks (p=0.75) did not differ before and after 2008.

Maternal and perinatal complications

Maternal and perinatal complications of all study pregnancies are presented in Table 3. In total, there were three twin pregnancies. A placental abruption occurred in one pregnancy in a patient with SLE without aPL. From the preterm births (<37 weeks), 44.2% occurred spontaneously and in the others labour was induced. Main indications for preterm induction of labour (<37 weeks) were HD (54.1%) and IUFD (12.5%). Of all pregnancies, 32.7% ended before 37weeks and 24.3% before 36 weeks. Of all live born infants, 55.3% was admitted to the medium care or neonatal intensive care unit. No neonatal deaths occurred. Of two infants with neonatal lupus one had a congenital heart block and the other cutaneous lupus. The incidence of pregnancy complications did not differ between first (n=70) and consecutive (n=74) pregnancies, with severe HD occurring in both first and consecutive pregnancies in 18.6% and 17.6% respectively (p=0.88), preterm birth <37 weeks in 36.6% and 28.9%

Table 2: Disease activity before and during pregnancy and postpartum.

	Total (n=144)	SLE -aPL (n=117)	SLE + aPL (n=14)	SLE + APS (n=13)	p-value
SLEDAI <6 months before pregnancy	2 [0-4]	2 [0-4]	2 [0-4]	3 [2-4]	0.22
SLEPDAI 1st trimester	2 [0-2]	2 [0-2]	2 [0-2]	2 [0.5-2]	0.41
SLEPDAI 2nd trimester	2 [0-2]	2 [0-2]	2 [1-3]	2 [0.5-2]	0.74
SLEPDAI 3rd trimester	2 [0-2]	0 [0-2]	2 [2-4.5]	2 [0.5-5.5]	<0.01
SLEDAI <6 months postpartum	2 [0-4]	2 [0-4]	2 [0-5]	4 [2-5.5]	0.27
Any flare before, during pregnancy or postpartum	44/144 (30.6)	35/117 (29.9)	5/14 (35.7)	4/13 (30.8)	0.94
Severe flare before, during pregnancy or postpartum	5/144 (3.5)	5/117 (4.3)	0/14 (0)	0/13 (0)	1.00
Mild/moderate flare before, during pregnancy or postpartum*	40/144 (27.8)	31/117 (26.5)	5/14 (35.7)	4/13 (30.8)	0.73
<6 months before pregnancy	9/144 (6.3)	8/117 (6.8)	0/14 (0)	1/13 (7.7)	0.67
1st trimester	6/144 (4.2)	5/117 (4.3)	1/14 (7.1)	0/13 (0)	0.72
2nd trimester	14/144 (9.7)	9/117 (7.7)	3/14 (21.4)	2/13 (15.4)	0.11
3rd trimester	7/144 (4.9)	6/117 (5.1)	1/14 (7.1)	0/13 (0)	0.77
<6 months postpartum	20/144 (13.9)	17/117 (14.5)	2/14 (14.3)	1/13 (7.7)	0.91

Data depicted as median [interquartile range] or numbers (%)
*A woman can flare multiple times before or during pregnancy or postpartum.
SLE: systemic lupus erythematosus, aPL: antiphospholipid antibodies, APS: antiphospholipid syndrome, SLEDAI: SELENA SLE disease activity index, SLEPDAI: SLE disease activity index adjusted for pregnancy.

respectively (p=0.32), IUFD in 4.2% and 3.9% respectively (p=1.00) and SGA in 14.7% and 14.9% respectively (p=0.98).

The rates of maternal and perinatal pregnancy complications for the studied reproductive period per patient are presented in Figure 2.

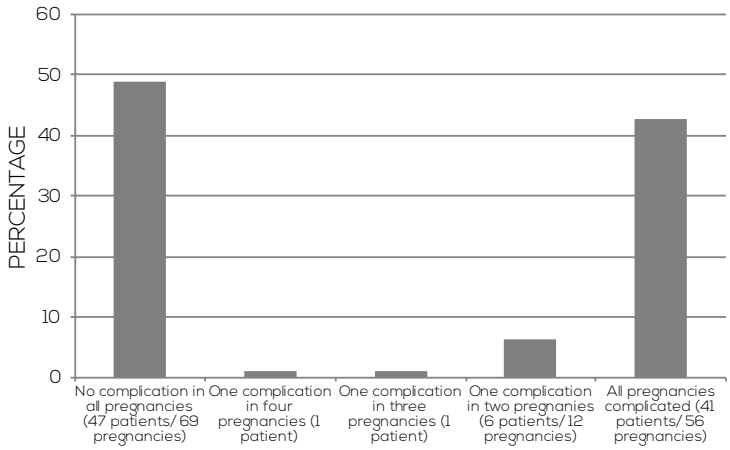
Live births

The mean number of live births was 1.7 ± 0.8 standard deviation (SD) per patient during the studied reproductive period. The mean number of pregnancies per patient was 2.4 ± 1.4 SD (including miscarriages) and miscarriage rate was 14% with a mean of 0.33 ± 0.7 SD per woman during the studied reproductive period.

Discussion

In this study we investigated disease activity and maternal and perinatal complications of ongoing pregnancies (>16 weeks) in patients with SLE in the Netherlands in a real-life setting. Low disease activity was found before, during and after pregnancy (mean SELENA SLEDAI/SLEPDAI scores of 2) in this population of patients with SLE. Median disease duration before the first study pregnancy was 7 years, irrespective of the presence of antiphospholipid antibodies. One exception was a median SLEPDAI score of 0 during the third trimester in the group without aPL. Still the incidence of maternal as well as perinatal complications was higher, especially preterm birth rate, compared to the general population regardless of the overall low disease activity.[26;27] The prevalences of severe HD, preterm birth, IUFD, SGA infants was similar in patients irrespective of antiphospholipid antibody status. One

Figure 2: Percentage of pregnancy complications during the studied reproductive period.



Complications included presence of any of the following: severe hypertensive disorders of pregnancy (including preeclampsia, eclampsia and HELLP-syndrome), placental abruption, preterm birth <37 weeks, intrauterine fetal death or small-for-gestational age infant.

exception was HELLP-syndrome, occurring more frequently in patients with SLE and APS. A striking finding is that the incidence of pregnancy complications in this population of patients with SLE did not decrease after the first pregnancy, as is seen in the general population. Another finding of interest is that the number of live births per women was 1.7.

In our patient population, only 6.3% experienced a mild flare before pregnancy, and no severe flares occurred. This might be a reflection of planned parenthood facilitated by the close collaboration between rheumatologists and gynecologists in our centers. The (on average) low disease activity before pregnancy likely contributed to the low flare rate during pregnancy of around 20%, which is comparable with other studies reporting incidence rates between 10-33%.[29-30] The incidence of postpartum flares (<6 months) in our cohort was amounting to 15%. However, patients with a flare during pregnancy were at greatest risk to develop a flare postpartum, and vice versa: 63.6% of flares postpartum occurred in patients with a flare before or during pregnancy. This finding calls for even more vigilance in the postpartum period especially in patients with increased disease activity during pregnancy.

The increase in use of HCQ after 2008 in the present study was neither associated with lower median disease activity scores during pregnancy, nor associated with a reduced incidence of pregnancy complications. This finding is partly in line with the results of a recent retrospective study which demonstrated no difference in flare rates or maternal pregnancy complications such as severe HD and IUFD between patients treated with and without HCQ.30 On the other hand, in this study a reduction of mild HD (PIH) and preterm birth <37 weeks was seen. Moreover, a prospective cohort study where HCQ was used in a similar number of pregnancies compared to the present study, and a small RCT suggested lower disease activity during pregnancy when HCQ was used.[16;31]

Maternal pregnancy complications occurred more often in the patients in our study compared to those reported in the general population, including mild and severe forms of HD and preterm

Table 3: Maternal and perinatal pregnancy complications in all study pregnancies.

Maternal complications					
Mild HD	21/144 (14.6)	18/117 (15.4)	1/14 (7.1)	2/13 (15.4)	0.82
Severe HD	26/144 (18.1)	19/117 (16.2)	3/14 (21.4)	4/13 (30.8)	0.32
Preeclampsia	24/140 (17.1)	18/113 (15.9)	3/14 (21.4)	3/13 (23.1)	0.82
Onset preeclampsia <34 weeks	8/24 (33.3)	7/18 (38.9)	1/3 (33.3)	0 (0)	1.00
Eclampsia	1/139 (0.7)	1/112 (0.9)	0/14 (0)	0/13 (0)	1.00
HELLP-syndrome	7/144 (4.9)	3/117 (2.6)	1/14 (7.1)	3/13 (23.1)	<0.01
Perinatal complications*	**N=147**	**N = 119**	**N = 15**	**N = 13**	
IUFD	6/147 (4.1)	6/119 (5.0)	0/15 (0)	0/13 (0)	1.00
Preterm birth (<37 weeks)	48/147 (32.7)	40/119 (33.6)	4/15 (26.7)	4/13 (30.8)	0.95
SGA infant	21/142 (14.8)	18/115 (15.7)	2/15 (13.3)	1/12 (8.3)	0.77
Neonatal lupus	2/147 (1.4)	2/119 (1.7)	0/15 (0)	0/13 (0)	1.00

Data depicted as numbers (%).
* There were three twin pregnancies.
SLE: systemic lupus erythematosus, aPL: antiphospholipid antibodies, APS: antiphospholipid syndrome, Mild HD: hypertensive disorders of pregnancy including pregnancy induced hypertension, Severe HD: hypertensive disorders of pregnancy including preeclampsia, eclampsia and HELLP (hemolysis elevated liver enzymes low platelets)-syndrome), IUFD: intrauterine fetal death, SGA: small-for-gestational age (birth weight <p10).

birth.[26;27] In the general population, HD affect about 5-10% of all pregnancies and preterm birth occurs in less than 10% of all pregnancies in developed countries.[26;27] The observed rate of HD in the present study, however, is in line with other studies.[6;32] The percentage of patients who developed HELLP-syndrome is low in our study with a significantly higher occurrence within the SLE+APS group compared to the other groups. We described that all patients with SLE+APS were treated with low-molecular-weight heparin during pregnancy.[25] This finding is in agreement with the perceived increased occurrence of HELLP-syndrome in patients with primary APS compared to mere aPL positivity in the literature.[33;34] This finding suggests an important but not exclusive role for antiphospholipid antibodies in the development of HELLP-syndrome.

The preterm birth rate was lower in the recent PROMISSE study, a prospective cohort study, compared to our study: 9% versus 24.3% <36 weeks gestation respectively.[18] This discrepancy might be explained by differences in the design of both studies. In the PROMISSE study, patients with important comorbidity, for example patients with diabetes mellitus or urinary protein–creatinine ratio greater than 1000 mg/g, and patients using medium or high dosages of glucocorticoids were excluded. Furthermore, the ethnic background of participants of both studies is different with 48% Caucasians in the PROMISSE study versus 83.5% in the present study. The preterm birth rate <37 weeks found in our study is in line with the results of other studies.[35;36]

To our knowledge, we compared for the first time the incidence of complications during the first and consecutive pregnancies. We demonstrated that incidences of HD, preterm birth <37 weeks, IUFD and SGA are similar for consecutive pregnancies compared to the first

pregnancy. This finding is not in line with observations in the general population, where nulliparity has been demonstrated as a risk factor for HD and multiparity reduces this risk, probably due to improvement of maternal-fetal immune adaptation in subsequent pregnancies.[37] We postulate that the maternal-fetal immune adaptation is different in patient with SLE. Patients with SLE should be informed about this finding in the preconceptional counseling. Moreover, we examined pregnancy complications during the studied reproductive period. Remarkably, almost half of the patients did not develop any severe complication during all of their pregnancies and more than 50% developed at least one severe complication during any of their pregnancies, also an item useful in the preconceptional counseling.

The mean number of pregnancies per woman in our study was 2.4 and resulted in a mean number of live births of 1.7 which is similar to results of a case-control study in the late nineties with a mean number of pregnancies of 2.3 and mean number of live births of 1.8.38 Limitation of that case-control study is that the severity of SLE was not described. A review examining pregnancy loss (not further defined) showed a decrease of pregnancy loss between 1960 and 2000.17 The results of our study implicate that no further improvement in the number of pregnancies and number of live births has occurred over the last 15 years, although it is unknown if our population (with a history of nephritis and thrombosis in 39.6% and 16.0% of the pregnancies respectively) is comparable with the population of Hardy et al considering the information given in the publication.[38]

The strength of the present study is that we did not use exclusion criteria with respect to disease activity, comorbidity, medication use and twin pregnancies which enables us to present pregnancy outcomes of a complete SLE population reflecting real-life setting. Furthermore, by including all pregnancies per woman during a 16 year period of time, we were able to examine pregnancy complications between first and consecutive pregnancies which, to our knowledge, has not been described before. A weakness of our study is that the majority of the population consisted of SLE patients without aPL which limits optimal comparison of women with aPL or APS.

In conclusion, this study provides an overview of SLE pregnancies over a 16-year period in two Dutch tertiary centers. Despite the finding of low disease activity before, during and after pregnancy in this population of patients with SLE and the absence of aPL in the majority of the patients, the incidence of maternal and perinatal complications was still higher compared to the general population. In the small subgroup of women with SLE and aPL or APS versus patients with SLE without aPL in our study, no differences in maternal and perinatal outcomes were found with exception for HELLP-syndrome. Of note, almost half of the women did not experience any complications during all of their pregnancies. A new finding is that incidence rates of severe HD, preterm birth <37 weeks, IUFD and SGA did not decrease in consecutive pregnancies compared to the first pregnancy. This observation is not in line with findings in the general population, underlining the risk for pregnancy complications in consecutive pregnancies in patients with SLE and the possible influence of the (auto)immune system on the maternal-fetal immune adaptation in SLE.

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3

Low-molecular-weight heparin and aspirin use in relation to pregnancy outcome in women with systemic lupus erythematosus and antiphospholipid syndrome: a cohort study

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Abstract

Objective: To relate anticoagulant use to pregnancy complications in women with SLE and primary APS.

Methods: All ongoing pregnancies, 184, in two Dutch tertiary centres between 2000-2015.

Results: LMWH and aspirin was prescribed in 15/109 SLE women without antiphospholipid antibodies (aPL), 5/14 with aPL, 11/13 with antiphospholipid syndrome (APS), 45/48 with primary APS. Main complications in the four treatment groups (no anticoagulant treatment, aspirin, LMWH, aspirin and LMWH) included hypertensive disorders of pregnancy (9.4%, 23.3%, 50%, 18.4% respectively, p=0.12) and preterm birth (16.7%, 34.3%, 75%, 36.8% respectively, p<0.001).

Conclusion: Maternal and perinatal complications occurred frequently, despite LMWH and aspirin use.

Introduction

Women with systemic lupus erythematosus (SLE) and antiphospholipid syndrome (APS) have an increased risk for pregnancy complications like hypertensive disorders of pregnancy (HD), preterm birth, intrauterine fetal death (IUFD) and small-for-gestational age (SGA) infants.[1] The risk for women with SLE is two to four times higher compared to women without SLE.[2;3] In 40% of SLE-patients, antiphospholipid antibodies (aPL) are found. Only after occurrence of thrombosis, IUFD, HD combined with birth <34 weeks gestation or consecutive spontaneous miscarriages, patients are diagnosed with APS (primary APS in patients without auto-immune disease and secondary APS in patients with auto-immune disease).[4;5] Secondary APS has been reported in approximately 14% of the SLE-patients.[6]

Despite lack of evidence from randomized controlled trials (RCTs) for the use of low-molecular-weight heparin (LMWH) to prevent pregnancy complications in women with SLE, it is often prescribed. Aspirin is also often prescribed in women with SLE and/or primary APS without evidence from an RCT, since it prevents recurrent HD in other populations.[7-10] To our knowledge, differences in pregnancy outcomes in relation to the use of LMWH and aspirin between women with SLE without aPL, SLE with aPL, SLE with APS and primary APS have not been well described yet. The current Dutch obstetric guideline advises LMWH for women with SLE and APS and aspirin may be considered for all women with SLE[11] Therapeutic advice of the Dutch guideline for treatment of women with primary APS is related to the cause of APS (e.g. thrombotic or obstetric complications).[12] The Royal College of Obstetrics and Gynaecology does not provide guidelines for these populations.

We have the opportunity to pool data from the rheumatology and obstetric departments of two tertiary centres during a 16-year period. The aim of this study is to describe LMWH and aspirin use during pregnancy in women with SLE and/or APS and the association with maternal and perinatal outcomes.

Specific questions are:

1. What are the anticoagulant treatment strategies used in women with SLE without aPL, SLE with aPL, SLE with APS and primary APS?
2. What is the incidence of maternal and perinatal complications in the four treatment groups: no anticoagulant treatment, aspirin only, LMWH only or both aspirin and LMWH?

This study is undertaken to provide more insight in anticoagulant therapy strategies in daily clinical practice and enhances our knowledge on the rationale of anticoagulant treatment in this specific population.

Methods

This cohort study was facilitated by a collaboration between two tertiary centres in the Netherlands performing joined care for pregnant women with SLE and/or APS by rheumatologists and obstetricians; VU University Medical Center in Amsterdam and University Medical Center Utrecht in Utrecht. All women with SLE and/or APS and an ongoing pregnancy (>16 weeks)

between 2000 and 2015 were included. Women were included when SLE or APS was diagnosed before pregnancy. The revised American College of Rheumatology criteria were used for the classification of SLE.[13] APS was diagnosed according to the Sapporo criteria.[4;5] Presence of aPL was based on two positive measurements of anticardiolipin antibodies, lupus anticoagulants or beta2-glycoprotein antibodies, measured at least 6 weeks or 12 weeks apart, neither during nor within ten weeks after pregnancy (when applicable).[4;5] Samples were considered positive for anticardiolipin antibodies or beta-2-glycoprotein antibodies when either above 40GPL or above the 99th percentile.

Data collection

Data were derived from similar databases in both centres recording all pregnancies of SLE- and/or APS-patients since 2000. First, the digital data program Mosos (BMA BV, Houten, the Netherlands) of both obstetric departments was used, which is a nationwide used program reporting deliveries above 16 weeks gestational age. A search was performed in this database for SLE, APS and aPL. The second database on SLE and/or APS and pregnancy was derived from both departments of Rheumatology, the Amsterdam longitudinal SLE cohort study at the VU University Medical Center and the SLE or APS and pregnancies database at the University Medical Center Utrecht. After identification of the participants, the medical charts were checked to obtain information. The Institutional Review Boards of both university hospitals concluded that official approval from a medical ethical committee was not needed due to the strictly observational character of this study.

Assessments

Collection of demographic and clinical data from medical charts included age, ethnicity, body mass index (BMI) and smoking habits. The medical charts also contained information about aPL status and year of diagnosis of SLE and/or APS. In VU University Medical Center, presence of anticardiolipin antibodies and lupus anticoagulant was collected. In the University Medical Center Utrecht, also data on the presence of beta2-glycoprotein antibodies were available in several cases. Information on the general history included lupus nephritis, arterial thrombosis, venous thrombosis, chronic hypertension and diabetes mellitus. Moreover, in women with SLE, occurrence of flares and thrombocyte count was assessed within six months before pregnancy.[14]

The information on the obstetric history included spontaneous miscarriages, HD (defined as preeclampsia, eclampsia or HELLP-syndrome), IUFD, placental abruption, preterm birth (defined as birth <37 weeks gestational age) and SGA infant (defined as birth weight <p10). Moreover, information about medication use during and after pregnancy, with specific notice of the use of LMWH and aspirin was collected. According to the protocol of both obstetric departments, it was strived to start treatment with LMWH and aspirin in the first trimester. Treatment with LMWH was classified into two doses; prophylactic and therapeutic dose.[15;16]

The information on maternal pregnancy complications included HD, placental abruption and preterm birth. Perinatal complications included IUFD, SGA infants and admission to neonatal intensive care unit (NICU) or medium care.

Statistics

Baseline characteristics were examined using descriptive statistics. Numbers and percentages of LMWH and aspirin usage per patient group were presented using descriptive statistics as well. For comparison of the four treatment groups concerning continuous variables, a linear regression analysis was performed, including a post-hoc analysis with Bonferroni correction for pairwise comparisons of treatment groups in case of a significant overall *p*-value for comparison of the four groups. To compare dichotomous variables, a logistic regression analysis was performed, also including a post-hoc analysis with Bonferroni correction. In these regression analyses we corrected for maternal age during pregnancy, BMI ≥ 25 kg/m^2 and chronic hypertension. If the total number of women with a complication was too low and therefore a logistic regression analysis could not be performed, a Fisher's exact test was performed. When the total number per patient group was sufficient, outcomes were compared between the four treatment groups using a Fisher's exact test, including a post-hoc analysis with Bonferroni correction.

Statistical analyses were performed with SPSS 22.0 (SPSS Inc, Chicago, IL, USA). A two-sided *p*-value inferior to 0.05 was considered to be statistically significant.

Table 1: Baseline characteristics per included pregnancy

	No LMWH or aspirin (n=66)	**Aspirin only (n=30)**	**LMWH only (n=12)**	**LMWH + aspirin (n=76)**
Number of women	47	19	8	50
General				
Age (years)	31.8 ± 4.1	32.5 ± 5.1	30.5 ± 3.8	32.1 ± 4.4
Non-caucasian	7/62 (11.3)	3/29 (10.3)	6/12 (50)	11/71 (15.3)
BMI (kg/m^2)	23.0 ± 3.6	!24.0 ± 3.9	24.2 ± 3.4	24.6 ± 5.3
Smoking	7/65 (10.8)	2/29 (6.9)	1/11 (9.1)	6/71 (8.5)
Obstetric history				
Nulliparous	33/66 (50.0)	13/30 (43.3)	6/12 (50.0)	28/76 (36.4)
Spontaneous miscarriages (at least one)	10/66 (15.2)	8/30 (26.7)	2/12 (16.7)	40/76 (52.6)
HD	5/27 (18.5)	7/17 (41.2)	2/5 (40.0)	17/50 (34.0)
IUFD	3/32 (9.4)	5/17 (29.4)	0/6 (0)	17/47 (36.2)
Placental abruption	0/35 (0)	1/17 (5.9)	0/6 (0)	5/50 (10.0)
Preterm birth	9/33 (27.3)	6/19 (31.6)	4/6 (66.7)	18/50 (36.0)
SGA	6/33 (18.2)	6/16 (37.5)	2/6 (33.3)	8/42 (19.0)
General history				
Lupus nephritis	20/66 (30.3)	16/30 (53.3)	5/9 (55.6)	14/55 (25.5)
Minor flare before pregnancy	2/64 (3.1)	1/30 (3.3)	1/8 (12.5)	1/33 (3.0)
Arterial thrombosis	1/66 (1.5)	3/30 (10.0)	0/12 (0)	12/76 (15.8)
Venous thrombosis	1/66 (1.5)	0/30 (0)	4/11 (36.4)	29/76 (38.2)
Hypertension	7/66 (10.6)	4/30 (13.3)	2/11 (18.2)	10/75 (13.3)
Diabetes	4/66 (6.1)	0/30 (0)	0/12 (0)	2/75 (2.7)
Time between onset SLE/APS and pregnancy (years)	7.9 ± 4.6	8.7 ± 5.5	5.3 ± 2.4	5.9 ± 5.3

LMWH: low-molecular-weight heparin, BMI: body mass index, HD: hypertensive disorders of pregnancy, IUFD: intra-uterine fetal death, SGA: small-for-gestational age (<p10), CDLE: chronic discoid lupus erythematosus. Data depicted as mean ± SD or numbers (%)

Table 2: Treatment with aspirin and/or LMWH during and after pregnancy per patient group.

	SLE without aPL (n=109)	SLE with aPl (n=14)		SLE with APS (n=13)		Primary APS (n=48)	
During pregnancy							
Aspirin only	28(25.7)	2	(14.3)	0	(0)	0	(0)
LMWH only	6* (5.5)	1	(7.1)	2	(15.4)	3	(6.1)
Prophylactic dose	3	1		1		-	
Therapeutic dose	2	-		1		3	
Aspirin and LMWH	15*(13.8)	5	(35.7)	11	(84.6)	45#	(93.8)
Prophylactic dose	13	5		6		25	
Therapeutic dose	1	-		5		18	
No treatment with aspirin or LMWH	60(55.0)	6	(42.9)	0	(0)	0	(0)
Postpartum							
LMWH use	29(29.0) 10(	10/ 14	(71.4)	9/ 11	(81.8)	46/ 46	(100)

* of 1 patient the dose of LMWH was unknown, # of 2 patients the dose of LMWH was unknown.
SLE: systemic lupus erythematosus, aPL: antiphospholipid antibodies, APS: antiphospholipid syndrome, LMWH: low-molecular-weight heparin. Data depicted as numbers (%)

Results

The inclusion criteria were met by 124 women with 184 pregnancies; 51 women with 75 pregnancies at the VU University Medical Center, 73 women with 109 pregnancies at the University Medical Center Utrecht. The baseline characteristics at the start of the pregnancy, including general and obstetric history, are depicted separately for each treatment group (Table 1). In six out of twelve pregnancies in the LMWH only group the ethnicity was non-Caucasian. These six pregnancies occurred in four women. SLE disease activity was low before pregnancy in 126 out of 136 SLE pregnancies, major flares did not occur, minor flares occurred in five pregnancies and disease activity before pregnancy was unknown in five pregnancies. None of the women with SLE did have a thrombocyte count below 100 before pregnancy. Hydroxychloroquine was used during pregnancy in 22/66 of the women treated without anticoagulant treatment, 14/30 treated with aspirin without LMWH, 0/12 treated with LMWH without aspirin and in 18/76 of the women treated with both aspirin and LMWH.

Anticoagulant treatment strategies used during pregnancy

ifferent anticoagulant treatment strategies in women with SLE without aPL, SLE with aPL, SLE with APS and primary APS are presented in vvvv. In the total population, LMWH was used in 47.8% and aspirin in 57.6% of the pregnancies. Aspirin was already used before pregnancy in nine women prior to 13 pregnancies (7.1% of all pregnancies). Three out of these nine women had a history of arterial thrombosis, in the other women the reason for chronic aspirin use was unknown.

History of women who used LMWH are depicted in Table 3 per patient group.

In the group SLE without aPL in two pregnancies the dose and reason for LMWH was unknown. In five pregnancies in this patient group, no LMWH was used despite previous venous thrombosis.

In the group with primary APS in two pregnancies the dose of LMWH was unknown (history

Table 3: History of women including prophylactic and therapeutic dose of low-molecular-weight heparin per patient group[15,16]

	SLE without aPL		SLE with aPL		SLE with APS		Primary APS	
	Prophylactic dose	Therapeutic dose	Prophylactic dose	Therapeutic dose	Prophylactic dose	Therapeutic dose	Prophylactic dose	Therapeutic dose
Previous arterial thrombosis	-	n=1	-	-	-	n=1	-	n=3
Previous venous thrombosis	n=1	-	-	-	n=2	n=2	n=4	n=15
Both previous arterial and venous thrombosis	-	-	-	-	n=1	-	-	-
History of nephritis	n=2	n=1	n=4	-	n=2	n=2	-	-
Both previous venous thrombosis and history of nephritis	n=7	-	-	-	-	n=1	-	-
Primary APS solely	NA	NA	NA	NA	NA	NA	n=21	n=3
Unknown	n=6	n=1	n=2	-	n=2	-	-	-

SLE: systemic lupus erythematosus, aPL: antiphospholipid antibodies, APS: antiphospholipid syndrome, NA: not applicable.

of previous arterial thrombosis (n=1) and unknown (n=1)).

Incidences of maternal and perinatal complications

Incidences of maternal and perinatal complications in the four treatment groups (no anticoagulant treatment, aspirin only, LMWH only or both aspirin and LMWH) are depicted in Table 4. Women receiving anticoagulant treatment had the highest frequency of maternal and perinatal complications, whereas women without anticoagulant treatment had least complications. The overall prevalence of HD was 18.1%, placental abruption 1.1%, preterm birth 33.2%, IUFD 4.8%, SGA infant 17.4% and admission of the infant to NICU or medium care 52.4% (25.3% and 27.1% respectively). The delivery of one woman with primary APS occurred at 23 weeks gestational age due to HD, which resulted in the only neonatal death in this population. The analysis of SGA showed a quasi-separation of the data, caused by chronic hypertension; none of the women with chronic hypertension had a SGA infant. Sub-analysis without correction for chronic hypertension (only correction for maternal age during pregnancy and BMI ≥ 25 kg/m^2) did not show any difference; the *p*-value remained the same (p=0.39). Maternal and perinatal outcomes in the patient group of SLE without aPL were stratified according to treatment, which demonstrated that women with anticoagulant treatment experienced more maternal and perinatal complications compared to women without anticoagulant treatment (Table 5). The numbers of included patients in the other patient groups were too small to perform further statistical analysis.

Discussion

In our cohort any anticoagulant treatment (LMWH and/or aspirin) was prescribed in 45.0% of women with SLE without aPL, in 57.1% of women with SLE with aPL and in 100% of women with SLE with APS or primary APS. The treatment group with aspirin and LMWH consisted

Table 4: Maternal and perinatal outcomes per treatment group.

	No LMWH or aspirin (n=66/67)^		Aspirin only (n=30/31)^		OR	LMWH only (n=12)		OR	LMWH + aspirin (n=76/77)^		OR	*p-value*
Patient group												
SLE without aPL	n=60		n=28			n=6			n=15			
SLE with aPL	n=6		n=2			n=1			n=5			
SLE with APS	n=0		n=0			n=2			n=11			
Primary APS	n=0		n=0			n=3			n=45			
Maternal complications												
HD	6/64	(9.4)	7	(23.3)	3.32 (0.90 – 12.29)	6	(50.0)	6.42 (1.26 – 32.79)	14	(18.4)	2.45 (0.78 – 7.71)	0.12
Placental abruption	0	(0)	0	(0)		0	(0)		2	(2.7)		0.70
Preterm birth	11	(16.7) *□β	13	(43.3)*	5.05 (1.81 – 14.14)	9	(75.0)□*	24.54 (4.36 – 138.2)	28	(36.8)*β	3.52 (1.46 – 8.49)	<0.001
Perinatal complication												
IUFD	1	(1.5)	2	(6.5)	4.55 (0.40 – 52.21)	2	(16.7)	13.20 (1.09 – 159.4)	4	(5.2)	3.57 (0.39 – 32.73)	0.10
SGA	12/66	(18.2)	2/29	(6.9)	0.48 (0.10 – 2.39)	4/11	(36.4)	2.30 (0.49 – 10.82)	13/72	(18.1)	1.46 (0.56 – 3.85)	0.39
Admission NICU or medium care	25/63	(39.7)*	21/28	(75.0)*	4.31 (1.55 – 12.00)	7/10	(70.0)	2.82 (0.62 – 12.76)	36/69	(52.2)	1.44 (0.68 – 3.06)	0.035

^ There were three twin pregnancies; number is different for neonatal outcomes, * significant difference between treatment with aspirin only and no anticoagulant treatment, βsignificant difference between treatment with LMWH only and no anticoagulant treatment, βsignificant difference between treatment with LMWH and aspirin and no anticoagulant treatment, ° significant difference between treatment with LMWH only and treatment with LMWH and aspirin, βsignificant difference between treatment with aspirin only and treatment with LMWH and aspirin.
LMWH: low-molecular-weight heparin, OR: odds ratio, HD: hypertensive disorders of pregnancy, SGA: small-for-gestational age infant (weight <p10), NICU: neonatal intensive care unit.
The analysis of SGA showed a quasi-separation of the data, caused by chronic hypertension; none of the women with chronic hypertension had a SGA child. Sub-analysis without correction for chronic hypertension did not show any difference in outcome; the p-value remains the same. Shown OR depict the analysis without correction for chronic hypertension.
Data depicted as mean ± SD or numbers (%) and Odds ratios (95% confidence interval).

mainly of women with SLE and APS or primary APS. The maternal and perinatal outcomes in the complete cohort showed that the subgroups with anticoagulant treatment experienced more maternal and perinatal complications compared to those without anticoagulant therapy. The same holds true for women with SLE without aPL. The overall incidence of maternal and perinatal complications was high, irrespective of treatment group and despite low SLE disease activity in the majority of the population within six months before pregnancy.

The value of anticoagulant treatment with LMWH and/or aspirin in women with SLE and/or APS is still under debate. Treatment with aspirin was recommended for all patients with SLE in a recent review article.[7] This is, amongst others, derived from studies finding a beneficial effect of aspirin in other populations at high risk for HD or intra-uterine growth.[10] Furthermore, treatment with LMWH in addition to treatment with aspirin was advised for women with either primary or secondary APS in review and overview articles[7;9;17;18] In women with SLE and aPL, authors of two retrospective studies suggested that LMWH treatment during pregnancy might be beneficial to reduce maternal and perinatal pregnancy complications.[19;20] However, in both studies all patients with SLE and aPL used LMWH, in absence of a control group without LMWH use.

An RCT investigating the effect of treatment with aspirin and LMWH in women with SLE is lacking and a limited number of RCTs on this subject in women with primary APS have been

Table 5: Maternal and perinatal outcomes in women with SLE without aPL.

	No LMWH or aspirin (n=60)		Aspirin only (n=28-29)^		LMWH only (n=6)		LMWH + aspirin (n=15-16)^		*p-value*
Maternal complications									
HD	4/58	(6.9)[¤]	7	(25.0)	4	(66.7)[¤]	4	(26.7)	0.001
Placental abruption	0	(0)	0	(0)	0	(0)	1	(6.7)	0.19
Pre-term birth	10	(16.7)*[ϕ¤]	13	(46.4)*	6	(100)[¤]	9	(60.0)[ϕ]	<0.001
Perinatal outcomes									
IUFD	1	(1.7)	2	(6.9)	2	(33.3)	1	(6.3)	0.022
SGA	10	(16.9)	2	(7.4)	3	(60.0)	2	(13.3)	0.052
Admission NICU or medium care	23/56	(41.0)*[ϕ]	20/26	(76.9)*	4/4	(100)	11/13	(84.6)[ϕ]	<0.001

^ There were two twin pregnancies; number is different for neonatal outcomes, * significant difference between treatment with aspirin only and no anticoagulant treatment, βsignificant difference between treatment with LMWH only and no anticoagulant treatment, βsignificant difference between treatment with LMWH and aspirin and no anticoagulant treatment, ° significant difference between treatment with LMWH only and treatment with LMWH and aspirin, βsignificant difference between treatment with aspirin only and treatment with LMWH and aspirin.
LMWH; low-molecular-weight heparin, HD; hypertensive disorders of pregnancy, SGA; small-for-gestational age infant (weight <p10). NICU: neonatal intensive care unit.
Data depicted as mean ± SD or numbers (%).

published.[21,22] To our knowledge, only two RCT's investigating the effect of LMWH on second and third trimester pregnancy complications in which (also) women with primary APS were included have been published: the TIPPS trial (n=22) and the FRUIT-RCT (n=32).[21,22] In the TIPPS trial women with thrombophilia were included. Subgroup analysis in women with primary APS showed no beneficial effect of LMWH compared to treatment without LMWH.[21] In the FRUIT-RCT, including only women with primary APS, also no beneficial effect of LMWH with aspirin was demonstrated compared to treatment with aspirin alone.[22]

The exact mechanism of action of LMWH and aspirin in the prevention of pregnancy complications is still unclear and is probably not limited to the anticlotting mechanism.

LMWH probably has an early effect that may occur at cellular level, by decreasing trophoblast apoptosis and increasing the production of proteases involved in the trophoblast invasion of the maternal endometrium.[23,24] In vitro studies have shown an effect of LMWH on angiogenesis in the placental villi and show an influence on the dysregulation of soluble vascular endothelial growth factor.[25,26] Furthermore, an inhibiting effect of heparin on complement activation is reported, which could reduce the risk of pregnancy complications.[27-29] On the other hand, LMWH might have an adverse effect by increasing soluble fms-like tyrosine-kinase-1 (a splice variant of vascular endothelial growth factor receptor) which contributes to HD.[30]

Aspirin is thought to improve the trophoblastic invasion of the uterine spiral arteries and might subsequently improve the development and efficacy of the placenta probably due to thrombocyte aggregation inhibition and/or an anti-inflammatory working mechanism.[10,31] These results, however, have been found in experimental setting and it is unknown what the relevance is for clinical practice.

In the present study, women with anticoagulant treatment during pregnancy had most maternal and perinatal complications. Evaluating our results, we suppose this finding is most likely explained by confounding by indication. Physicians did not prescribe LMWH and/or aspirin

in cases with a perceived low a priori risk for complications, concerning both obstetrical and SLE and/or APS history. Moreover, SLE is a complex multi-organ autoimmune condition and poor obstetric outcomes are probably not only caused by thrombotic mechanisms but also influenced by disease activity and renal function before pregnancy.[32;33] On the other hand, as stated before, the mechanisms of action of both LMWH and aspirin are probably not limited to the anticlotting mechanism. In all treatment groups, prevalences of complications were high when compared to healthy women. The results of our study, including a high prevalence of HD, preterm birth and admission of the neonate in patients with SLE or APS, are in line with other studies.[2;3;34-36]

Beneficial effects of treatment with LMWH to prevent second and third trimester obstetric complications in women with SLE have not been reported. Approximately 2% of the pregnancies with daily LMWH injections are complicated by significant bleedings either antepartum or postpartum.[16] To further develop evidence-based guidelines for LMWH use during pregnancy in women with SLE, a multicentre RCT is needed to examine the possible effect of LMWH in addition to treatment with aspirin.
Likewise, in women with primary APS a beneficial effect of LMWH on second and third trimester pregnancy outcomes has not been proven.[21;22] For women with APS and a thrombotic event in history, treatment with LMWH during pregnancy is recommended, since LMWH has been reported to be effective in preventing venous thrombosis in pregnancy.[16]
Presently, aspirin is advised in all women with SLE and/or (primary) APS with the drawback of lack of knowledge on the exact working mechanism and the absence of an underlying RCT. Due to limited side-effects and proven beneficial effects in other high-risk populations, the recommendation to use aspirin in the prevention of pregnancy complications was extended to women with SLE and primary APS.[7;10]

To our knowledge, this is the first study presenting an overview of anticoagulant treatment in daily clinical practice in women with SLE and/or APS comparing pregnancy outcomes between different anticoagulant treatment groups. We were able to describe a considerable number of pregnancies in women with either SLE or primary APS in two tertiary centres performing joined care for pregnant SLE and APS women by rheumatologists and obstetricians in a 16-year period. The data on anticoagulant treatment, although retrospective, are valuable since there is a trend to prescribe LMWH and aspirin more frequently in patients with SLE nowadays. Therefore, comparison of the results of different treatment strategies on pregnancy outcomes might be more difficult in the future, since the number of pregnancies in which anticoagulant treatment is not applied will probably decrease. Moreover, analysis in relation to maternal and perinatal outcomes per treatment modality has not been published yet. Furthermore, anticoagulant treatment was started in the first trimester, which is important since in other populations aspirin use has been proven to be beneficial in the prevention of pregnancy complications if initiated before 16 weeks gestational age.[10] Limitations of our study are the nature of the retrospective set-up making missing data inherent. In addition, the number of women in the treatment group with LMWH is small despite an observation

period of 16 years, which impairs the ability to compare pregnancy outcomes in this subgroup with those in the other treatment groups.

In conclusion, this study provides an overview of LMWH and aspirin use in daily clinical practice in a population of women with SLE and/or APS. Pregnancy complications were frequent irrespective of treatment group. Women treated with anticoagulants showed even a higher frequency of maternal and perinatal complications compared to women without anticoagulant treatment, likely confounded by indication.

With the present knowledge, use of LMWH should be limited to women with a history of venous thrombosis. To examine the additional beneficial effect of LMWH besides aspirin on pregnancy outcome in women with SLE without a history of venous thrombosis, a randomized controlled trial is needed. Prescription of aspirin is advised for all pregnant women with SLE and/or (primary) APS.

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Low-molecular-weight heparin for prevention of placenta-mediated pregnancy complications: protocol for a systematic review and individual patient data meta-analysis (AFFIRM)

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Abstract

Background: Placenta-mediated pregnancy complications include pre-eclampsia, late pregnancy loss, placental abruption, and the small-for-gestational age newborn. They are leading causes of maternal, fetal, and neonatal morbidity and mortality in developed nations. Women who have experienced these complications are at an elevated risk of recurrence in subsequent pregnancies. However, despite decades of research no effective strategies to prevent recurrence have been identified, until recently. We completed a pooled summary-based meta-analysis that strongly suggests that low-molecular-weight heparin reduces the risk of recurrent placenta-mediated complications. The proposed individual patient data meta-analysis builds on this successful collaboration. The project is called AFFIRM, An individual patient data meta-analysis oF low-molecular-weight heparin For prevention of placenta-medIated pRegnancy coMplications.

Methods/Design: We conducted a systematic review to identify randomized controlled trials with a low-molecular-weight heparin intervention for the prevention of recurrent placenta-mediated pregnancy complications. Investigators and statisticians representing eight trials met to discuss the outcomes and analysis plan for an individual patient data meta-analysis. An additional trial has since been added for a total of nine eligible trials. The primary analyses from the original trials will be replicated for quality assurance prior to recoding the data from each trial and combining it into a common dataset for analysis. Using the anonymized combined data we will conduct logistic regression and subgroup analyses aimed at identifying which women with previous pregnancy complications benefit most from treatment with low-molecular-weight heparin during pregnancy.

Discussion: The goal of the proposed individual patient data meta-analysis is a thorough estimation of treatment effects in patients with prior individual placenta-mediated pregnancy complications and exploration of which complications are specifically prevented by low-molecular-weight heparin.

Systematic review registration: PROSPERO (International Prospective Registry of Systematic Reviews) 23 December 2013, CRD42013006249

Background

Placenta-mediated pregnancy complications include preeclampsia (PE), late pregnancy loss, placental abruption and the small-for-gestational age (SGA) newborn. We completed a pooled summary-based meta-analysis that strongly suggests that low-molecular-weight heparin (LMWH) reduces the risk of placenta-mediated complications in subsequent pregnancies.[1]

A successful pregnancy requires the development of adequate placental circulation. It has been hypothesized that thrombosis in the placental bed is at least partially responsible for placenta-mediated pregnancy complications.[2-4] It has also been suggested that these complications are the result of abnormal placental development with underdeveloped placental vasculature or placental inflammation.[5,6] These complications represent an important health problem because they are common, affecting more than one in six pregnancies,[7] and often have a devastating outcome for the affected women, their unborn children, their families, and society. Specifically, PE (characterized by a new onset of elevated blood pressure and proteinuria during pregnancy) is one of the most common causes of maternal mortality in the developed world.[8-11] SGA newborns often suffer longterm effects including developmental delay, poor school performance, and a significantly lower likelihood of academic and professional success.[12-14] Fetal loss is a devastating event for pregnant women and their families. Placental abruption (separation of the placenta from the uterus before birth) can, in the most severe cases, lead to maternal hemorrhage with the risk of transfusion and both maternal and fetal death.

The risk of recurrent placenta-mediated pregnancy complications in subsequent pregnancies is substantial. For example, women with prior severe PE will have a 25 to 65% risk of recurrent PE, a 3% risk of placental abruption, and a 10% risk of SGA (<10th percentile).[15,16] These complications may be multiple (for example both PE and SGA) and not isolated to the placenta-mediated complication experienced in a prior pregnancy.15,17 There are no highly effective preventative strategies that can be used in subsequent pregnancies. Aspirin offers small relative risk reductions in patients with prior PE and SGA, however, it may be more effective at reducing risk (approximately a 40% reduction) if started early in the pregnancy (before 16 weeks).[18,19] There are no proven preventative strategies for the other complications. It has been postulated that anticoagulants might prevent placenta-mediated pregnancy complications by reducing placental thrombosis and/or affecting maternal coagulation activation or inflammation. Recent randomized controlled trials (RCTs) conducted to determine if LMWH can prevent recurrent placenta-mediated pregnancy complications suggest an important treatment effect,[20-24] but this finding has not been universal.[25]

Although it appears that LMWH is a promising therapy in the prevention of placenta-mediated pregnancy complications, there are disadvantages to the premature adoption of this intervention without sufficient evidence of benefit. If LMWH is used universally for all women with prior placenta-mediated pregnancy complications, we may be intervening

unnecessarily and exposing women to a risk of undesirable and potentially fatal, albeit rare, side effects (major bleeding, heparin-induced thrombocytopenia, osteoporotic fractures, withholding of epidural analgesia due to fear of causing epidural hematoma, and paralysis).[26,27] Less serious side effects including skin reactions, minor bleeding, and transient elevations in liver enzymes are more commonly experienced.[28,29] Therapy is also associated with cost and inconvenience since the drug is expensive and is administered by injection either once or twice a day. Therefore, it is necessary to answer the question as to who benefits from LMWH prophylaxis during pregnancy and to determine the nature and magnitude of these benefits more precisely. The individual patient data meta-analysis (IPDMA) has the potential to answer these important questions and determine the risk/benefit ratio of therapy for various subgroups of women.

The composite outcome, including all placenta-mediated pregnancy complications, that is used in many RCTs is heterogeneous and not all individual outcomes can be considered equally serious in terms of potential consequences for the mother and newborn. For example, late term pre-eclampsia is clinically less worrisome since the symptoms tend to be less severe and generally resolve with delivery. Conversely, women who develop pre-eclampsia earlier in the pregnancy have more serious clinical consequences including a greater risk of maternal and neonatal death. Our pooled summary meta-analysis suggests that LMWH may prevent severe pre-eclampsia and early preeclampsia with less of an effect on late onset pre-eclampsia.[1] Confirmation of these findings is extremely important for clinicians treating these women and has direct relevancefor clinical practice worldwide.

There are many challenges associated with recruiting pregnant women to RCTs with a drug intervention including: the biases of clinicians either for or against the therapy (based on insufficient evidence of benefit and lack of knowledge about potential risk); the concerns of the pregnant woman and her family about the health and safety of the mother and baby; and the demands during pregnancy of attending additional appointments and investigations associated solely with study participation.[1] Furthermore, the pharmaceutical industry often excludes pregnant women from trials due to liability concerns. As a result, there is a dearth of RCTs evaluating LMWH in this population compared to other patient groups (such as oncology or orthopedic surgery). Those RCTs that do exist are all academically driven and may not have the same financial and human resources that are available to trials that are sponsored by the pharmaceutical industry. Therefore, meta-analysis is an essential tool that allows for greater statistical power by pooling the existing small RCTs evaluating LMWH for the prevention of placenta-mediated pregnancy complications.
Our recent pooled summary-based meta-analysis of six RCTs (Table 1) included 848 pregnant women with a history of pre-eclampsia, a SGA neonate (<10th percentile), placental abruption, or late pregnancy loss (more than 12 weeks gestation) in a previous pregnancy.[1] The primary finding was that 67 out of 358 (18.7%) women taking LMWH during pregnancy had recurrent severe placentamediated pregnancy complications, as compared with 127 out of 296 (42.9%) women with no LMWH (relative risk eduction 48% (95% CI 14 to 68%; (I2

Table 1: Previously identified trials that meet the inclusion criteria for AFFIRM

Study Name & First Author	Year	Country & Sample Size	Participants	Intervention & Control	Outcomes	Commitment to Participate in IPDMA
TIPPS[30]* Rodger	2013	Canada, Multinational N = 292	Thrombophilia + previous high risk criteria	Dalteparin 5000 IU to 20 wks then 10,000 IU to 36 wks **vs** no Dalteparin	PE, SB, abruption, SGA <10th percentile	Yes
FRUIT[20] de Vries	2012	Netherlands, Multinational N = 139	Prior early onset PE (n = 107) and/or SGA <10th percentile (n = 94)	Dalteparin 5000 IU + ASA **vs** ASA	PE prior to 34 weeks GA	Yes
HAPPY[25] Martinelli	2012	Italy, Multi-center N = 135	Prior PE (n = 52), prior loss >15 weeks (n = 49), prior SGA <10th percentile (n = 28) or prior abruption (n = 5)	Nadroparin 3800 IU **vs** no Nadroparin	PE, Loss >15 weeks GA, SGA <10th percentile and/or abruption	Yes
NOH-PE[21] Gris	2011	France, Single center N = 224	Prior severe PE (n = 224)	Enoxaparin 4000 IU + ASA **vs** ASA	PE, SB, abruption, SGA <5th percentile	Yes
NOH-AP[24] Gris	2010	France, Single center N = 160	Prior abruption (n = 160; 70 with PE)	Enoxaparin 4000 IU+/-ASA **vs** +/- ASA	PE, SB, abruption, SGA <5th percentile	Yes
Rey[23]	2009	Canada, Multi-center N = 116	Prior early PE (n = 60),prior abruption (n = 16),prior SGA <5th percentile (n = 21),loss >12 weeks (n = 17)	Dalteparin 5000 IU+/-ASA **vs** +/- ASA	PE, SB, abruption, SGA <5th percentile	Yes
Mello[22]	2005	Italy, Single center N = 80	Prior PE with ACE DD (n = 80)	Dalteparin 5000 IU **vs** no Dalteparin	PE, SGA <10th percentile	Unable to contact

ASA = aspirin; GA = gestational age; IPDMA = individual patient data meta-analysis; IPDMA = individual patient data meta-analysis; PE = pre-eclampsia; RCT = randomized controlled trial; SB = stillbirth; SGA = small-for-gestational age.
Trial Names:
TIPPS = Thrombophilia In Pregnancy Prophylaxis Study *accepted for publication in the Lancet
FRUIT = FRactionated heparin in pregnant women with a history of Utero-placental Insufficiency and Thrombophilia
NOH-AP = Nîmes Obstetricians and Haematologist – abruptio placentae
NOH-PE = Nîmes Obstetricians and Haematologist - pre-eclampsia
HAPPY = Heparin in pregnant women with Adverse Pregnancy outcome to improve the rate of successful PregnancY

69%). However, since the meta-analysis results apply to a heterogeneous group of women with a mixture of placenta-mediated pregnancy complications of varying prior severity and the primary outcome for the meta-analysis was a composite of all placenta-mediated complications (also of varying severity), it is not clear which subgroups of women derive the most benefit from LMWH (which outcomes are reduced and which severity of outcomes are impacted). Before recommendations for clinical practice can be advocated, it is necessary to conduct more detailed analyses of the existing data to determine potential benefits for subgroups of women, to adjust for important baseline characteristics of participants, and to explore other treatment-related reasons for the reported heterogeneity (for example specific LMWH drug (dalteparin, nadroparin or enoxaparin), LMWH dose, gestational age when drug was initiated, and co-interventions such as concomitant ASA use).

IPDMA has been proposed as an advantageous methodological approach when subgroup analyses are hypothesized to be clinically relevant. Analyzing original data from individual patients makes use of a much richer dataset and has greater statistical power than conventional metaanalysis.[31,32]

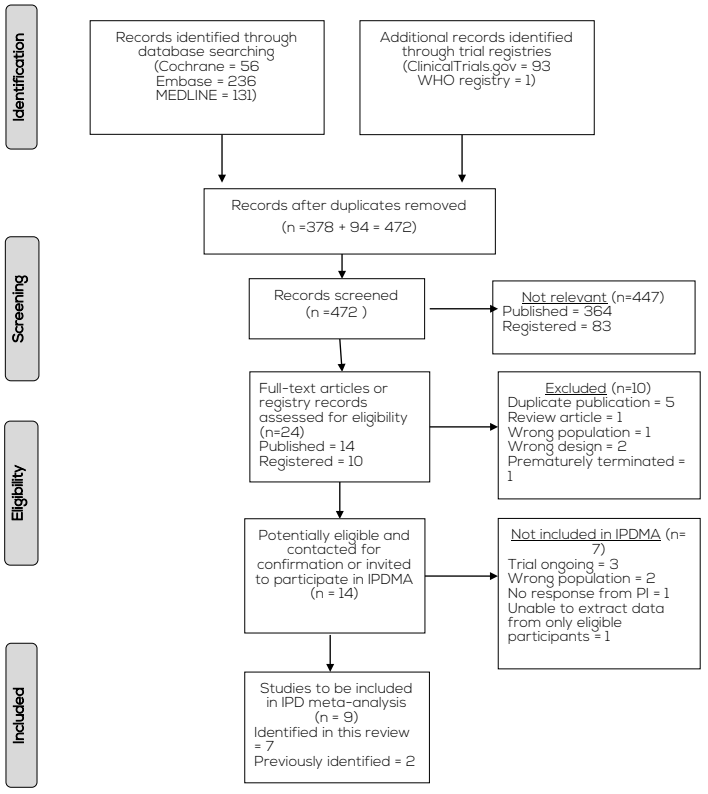
Furthermore, for this project, IPDMA will allow for adjustment for covariates that are known to be important in the recurrence of placenta-mediated pregnancy complications. Such an analysis will also enable us to explore clinical, methodological, and statistical heterogeneity more robustly. IPDMA is an attractive method to answer our study questions since it 'dramatically and consistently' has more power to detect interactions between risk groups.[33]

Methods/Design

Research questions

The primary research question is: Which women with previous placenta-mediated pregnancy complications have a reduction in the risk of future complications when treated with LMWH during pregnancy? Secondary research questions are: Which of the placenta-mediated pregnancy complications are avoided? Are severe and/or early onset or non-severe and/or late onset complications avoided? Does LMWH cause major bleeding in women with prior placenta-mediated pregnancy complications? And, are any other side effects increased by LMWH use in women with prior placenta-mediated pregnancy complications (thrombocytopenia, osteoporotic fractures or allergic reactions)?

Figure 1: PRISMA flow diagram of AFFIRM's systematic review



The proposed project is called AFFIRM (An individual patient data meta-analysis oF low-molecular-weight heparin For prevention of placenta-medlated pRegnancy complications), PROSPERO registration number: CRD42013006249. We will synthesize individual patient data from RCTs of LMWH for the prevention of recurrent placenta-mediated pregnancy complications. The overall objective of the meta-analysis is to directly inform clinical practice and the development of clinical practice guidelines. The study is coordinated by the Clinical Epidemiology Program at the Ottawa Hospital Research Institute. Conceptually, the research approach involves four sequential phases: a systematic review, knowledge synthesis planning, data extraction and analysis, and interpretation of results and knowledge translation. The first two phases have been completed and are therefore described below in the past tense. No data have been extracted or recoded for the common dataset and no statistical analyses have been performed; these steps are outlined in the future tense.

Systematic review

Electronic search strategies were developed and tested through an iterative process by an experienced medical information specialist in consultation with the review team. The strategy was peer-reviewed prior to execution by an experienced information specialist using the Peer Review of Electronic Search Strategies (PRESS) checklist.[34] The following search was conducted in May 2013: using the OVID platform, we searched Ovid MEDLINE™, Ovid MEDLINE™ In-Process & Other Non-Indexed Citations, and EmbaseClassic + Embase (strategy included as Additional file 1). We also searched the Cochrane Library on Wiley (including CENTRAL, Cochrane Database of Systematic Reviews, DARE, and HTA). ClinicalTrials. gov and the WHO International Clinical Trials Registry were searched to identify relevant in-process and completed trials. Strategies utilized a combination of controlled vocabulary (such as 'hypertension, pregnancyinduced', 'placental insufficiency', 'heparin, low-molecularweight') and keywords (pre-eclampsia, abruption, and LMWH). Vocabulary and syntax were adjusted across databases. Animal studies were excluded but there were no language or date restrictions on any of the searches. We sought additional references through hand- searching the bibliographies of relevant items. Search results are summarized in a preferred reporting items for systematic reviews and meta-analyses (PRISMA) diagram (Figure 1) and details of potentially eligible trials are provided in Tables 2 and 3.

Inclusion criteria

RCTs with an LMWH intervention for the prevention of recurrent placenta-mediated pregnancy complications were eligible. The study population of interest included currently pregnant women with prior pregnancies complicated by one or more of the following: PE, placental abruption, SGA newborn (<10th percentile), pregnancy loss after 16 weeks gestation or two losses after 12 weeks gestation. The principal investigators of potentially eligible trials identified by the systematic review (see Tables 1, 2 and 3) were contacted via email to request additional information about the study population. Once eligibility was confirmed, investigators were invited to participate in the IPDMA and attend the AFFIRM project planning meeting. The lead investigators of the largest and most recently completed trials agreed to contribute

Table 2: Potentially eligible published trials identified by AFFIRM's systematic review

Study Name & First Author	Year	Country & Sample Size	Participants	Intervention & Control	Relevant Outcomes	Comment re: Inclusion in IPDMA
ETHIG II * Schleussner[43]	Abstract 2013	Germany N = 449	Recurrent pregnancy loss	Dalteparin 5000 IU + vitamins **vs** multivitamins	Intact pregnancy at 24 wks GA; PE; IUGR <5th percentile; abruption	Yes
Giancotti[38]	2012	Italy N = 167 (pregnant)	Recurrent pregnancy loss	Enoxaparin 40 mg **vs** Enoxaparin 40 mg + ASA **vs** ASA	Live births	Not eligible (All losses <12 weeks GA)
Salman[39]	Abstract 2012	Egypt N = 150	Recurrent pregnancy loss	Tinzaparin 4500 IU **vs** folic acid	Continuation of pregnancy after 20 weeks	Not eligible (All women with early losses)
HABENOX[40] Visser	2011	Finland, Sweden, Netherlands N = 207	Women with recurrent early or late miscarriage	Enoxaparin 40 mg **vs** Enoxaparin 40 mg + ASA **vs** ASA	Live birth rate; PE; IUGR <2 SD; abruption	Yes
SPIN[35] Clark	2010	UK, New Zealand N = 294	Recurrent pregnancy loss	Enoxaparin 40 mg + ASA **vs** no drug	Pregnancy loss	GA of past losses not available centrally
ALIFE [41] Kaandorp	2010	Netherlands N = 299 (pregnant)	Recurrent pregnancy loss	Nadroparin 2850 IU + ASA **vs** ASA **vs** placebo	Pregnancy loss; SGA <10th percentile; PE; HELLP; abruption	Yes
HepASA[34] Laskin	2009	Canada N = 88 Terminated at interim analysis	Recurrent pregnancy loss	Dalteparin 5000 IU + ASA **vs** ASA	Live births	Unable to contact

ASA = aspirin; GA = gestational age; HELLP = HELLP syndrome (hemolysis, elevated liver enzymes, low platelet count); IPDMA = individual patient data meta-analysis; IUGR = intrauterine growth restriction; PE = pre-eclampsia; SB = stillbirth; SGA = small-for-gestational age.
Trial titles:
SPIN = Scottish Pregnancy Intervention Study HepASA = Low Molecular Weight Heparin and Aspirin in the Treatment of Recurrent Pregnancy Loss
ALIFE = Anticoagulants for Living Fetuses
HABENOX = Low Molecular Weight Heparin and/or Aspirin in Prevention of Habitual Abortion
ETHIG II = Effectiveness of Dalteparin Therapy as Intervention in Recurrent Pregnancy Loss *final results in preparation for publication.

individual patient data to this collaboration. Data from two small trials[22,41] were not included because the investigators did not respond; in one of these trials only a small proportion of the total study population would have been eligible to contribute data to AFFIRM.[41] Some of the women in the Scottish Pregnancy Intervention Study (SPIN) trial would have been eligible for inclusion in AFFIRM, however,the trial database does not include sufficient detail about the timing of previous pregnancy losses to determine the eligibility of individual participants.[39]

Knowledge synthesis planning

A crucial step in the success of the project was the development of the knowledge synthesis and knowledge translation plans. A full-day review team meeting was held in Amsterdam on 4 July 2013. The purpose was to allow for extensive discussion and consensus-reaching on important study variables and outcomes and to consider strategies for merging the existing datasets in a centralized database. Participants included the principal investigators of the included RCTs and statisticians with in-depth knowledge of the trial data. The principal

Table 3 Potentially eligible registered trials identified by AFFIRM's systematic review

Study Name & Principal Investigator	Identified Through	Participants	Intervention & Control	Outcomes	Comment re: Inclusion in IPDMA
EPPI McLintock	Ongoing RCT (New Zealand) ANZCTR registry ACTRN12609000699268	Prior PE or SGA	Enoxaparin 40 mg **vs** standard care	PE, SGA	Recruitment ongoing
HEPEPE Haddad	Ongoing RCT (France) Clinicaltrials.gov NCT00986765	Prior severe pre-eclampsia	Enoxaparin 4000 IU + ASA **vs** ASA	PE; IUGR; abruption; perinatal death	Recruitment ongoing
HOPPE Llurba	Ongoing RCT (Spain) Clinicaltrials.gov NCT01388322	Prior severe PE, SGA, loss, or abruption	Enoxaparin 40 mg or 80 mg (weight-based) **vs** no intervention	PE; IUGR; abruption; fetal death	Recruitment ongoing

ASA = aspirin; IPDMA = individual patient data meta-analysis; IUGR = intrauterine growth restriction; PE = pre-eclampsia; SGA = small-for-gestational age.
Trial names:
EPPI = Enoxaparin for the Prevention of Preeclampsia and Intrauterine growth restriction
HEPEPE = Prevention of Maternal and Perinatal Complications by Enoxaparin in Women With Previous Severe Preeclampsia (original title is French)
HOPPE = Low Weight Heparin prOphylaxis for Placental Mediated Complications of PrEgnancy

investigators are all practicing clinicians (obstetricians and hematologists) who are also knowledge users in this clinical area.

Outcome measures

The detailed definitions for the IPDMA outcomes were agreed upon by investigator consensus at the face-to-face meeting. The definitions and diagnostic criteria for each outcome variable are documented in a data dictionary and the research protocol. These definitions, which have been reviewed by all investigators, allow standardization across studies and decrease the potential for bias.

AFFIRM's primary outcome is a composite outcome including four pregnancy complications: early-onset or severe pre-eclampsia, birth of a small-for-gestational age newborn with a birth weight <5th percentile, placental abruption, and late pregnancy loss. To qualify as a primary outcome event, the pregnancy complication must satisfy one or more predefined criteria. Early onset pre-eclampsia is diagnosed at less than 34 weeks' gestation. Severe pre-eclampsia is characterized by at least one criterion indicative of severe disease; these are, a systolic blood pressure ≥ 160 mm Hg or diastolic blood pressure ≥110 mm Hg, proteinuria > 0.5 g/24 hours, elevated liver enzymes (more than two times the local upper range of normal), platelets < 100 × 109/L, pulmonary edema, seizures (eclampsia), headache or other neurological manifestations (stroke, intracranial hemorrhage, cerebral edema, hyperreflexia, and visual impairment), coagulopathy, oliguria (<30 ml/hr) or HELLP syndrome (hemolysis, elevated liver enzymes, low platelet count). Birth of a small-for-gestational age newborn with a birth weight <5th percentile is determined using local gender and gestational age specific birth weight charts. The placental abruption outcome requires a clinical diagnosis of placental abruption leading to delivery. A late pregnancy loss occurs at or after 20

weeks of gestation and cannot be explained by other factors, including fetal chromosomal abnormalities, maternal infection, cervical insufficiency or incompetence, or an intentional termination of the pregnancy.

Nineteen secondary outcomes have been defined for AFFIRM, including the four individual components of the primary outcome: severe or early-onset pre-eclampsia, birth of a small-for-gestational age newborn <5th percentile, placental abruption and late pregnancy loss, all as outlined above. Pre-eclampsia (non-severe) is characterized by a systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg and proteinuria >0.3 g/24 hours. A diagnosis of HELLP syndrome required 3 criteria, hemolysis [lactate dehydrogenase (LDH) > 600 IU/L or serum bilirubin >1.2 mg/dl] an abnormal elevation of liver enzymes (more than two times the local upper range of normal), and platelets <100 × 109/L. Preterm delivery <34 weeks and < 37 weeks are pre-specified outcomes. A perinatal loss is any fetal or neonatal death at over 20 weeks gestational age and less than or equal to 28 days post-partum and neonatal mortality is considered any neonatal death after birth and less than or equal to 28 days post-partum. Birth of a small-for-gestational age newborn <10th percentile is determined based on local gender and gestational age specific birth weight charts.

Adverse maternal outcomes include thrombocytopenia, defined as a platelet count <75,000 × 109/L, and bleeding outcomes at various time points. Antepartum major bleeding is defined using the criteria proposed by the International Society on Thrombosis and Haemostasis (ISTH).[42] That is, clinical or radiological evidence of bleeding with at least one of the following criteria: associated with a fall in hemoglobin of 2 g/dL (1.24 mmol/L) or more; or a requirement for transfusion of two or more units of red blood cells or whole blood; or symptomatic bleeding occurring in a critical site: intracranial, intraspinal, intraocular, pericardial, intra-articular, intramuscular with compartment syndrome, or retroperitoneal, or was considered to have contributed to maternal death. Peripartum major bleeding is hemorrhage occurring after the onset of labour or start of surgical delivery and within 24 hours postpartum that meets at least one of the following: necessitating a surgical procedure, or associated with a fall in hemoglobin of 4 g/dL (2.48 mmol/L) or more, or a requirement for transfusion of two or more units of red blood cells or whole blood, or estimated peripartum blood loss >1000 ml, or considered to have contributed to maternal death. Peripartum minor bleeding is hemorrhage occurring after the onset of labour or start of surgical delivery and within 24 hours postpartum that does not meet any criterion above and with estimated peripartum blood loss between 500 and 1000 ml. Postpartum major bleeding is clinical or radiological evidence of bleeding occurring between 24 hours and 6 weeks postpartum and meeting at least one of the following ISTH criteria: associated with a fall in hemoglobin of 2 g/dL (1.24 mmol/L) or more, or a requirement for transfusion of two or more units of red blood cells or whole blood, or symptomatic bleeding occurring in a critical site: intracranial, intraspinal, intraocular, pericardial, intraarticular, intramuscular with compartment syndrome, or retroperitoneal, or considered to have contributed to maternal death

An allergic reaction to LMWH is a reaction following the administration of LMWH that results in anaphylaxis or a rash requiring discontinuation of the allocated LMWH. Heparin-

induced thrombocytopenia (HIT) is defined as a clinical diagnosis of HIT and a minimum of a positive PF4 HIT ELISA assay. The venous thromboembolism outcome includes deep vein thrombosis (DVT) and/or pulmonary embolism. The criteria for diagnosis of DVT are venography demonstrating a constant intraluminal filling defect in the deep veins above the trifurcation of the popliteal vein or compression ultrasound revealing a non-compressibility of a venous segment above the trifurcation of the popliteal vein. Diagnosis of distal, below the knee DVT, is by either venography or compression ultrasound. Diagnostic criteria for pulmonary embolism are pulmonary angiography demonstrating a constant intraluminal filling defect or a cutoff of a vessel more than 2.5 mm in diameter, or ventilation/perfusion (V/Q scan) indicating high-probability, or pulmonary embolism found at autopsy.

Extraction and recoding of individual patient data

The definitions for each variable to be included in AFFIRM's common dataset are documented in a data dictionary to allow standardization across studies and decrease the potential for misclassification and bias. A template for the common dataset has been developed in Microsoft Excel and will be provided to the principal investigator of each included trial. Recoded anonymized individual patient data from each of the trials will populate the Excel template. The recoded datasets for each of the individual trials will be saved on an IronKey™ USB flash drive and sent by courier to the coordinating center in Ottawa.

The AFFIRM common dataset will include individual patient data in 10 pre-defined categories: administrative and demographic data, thrombophilia, maternal medical history, pregnancy history, current pregnancy and delivery, infant data, pre-eclampsia outcome, other outcome events, intervention and treatment during pregnancy, and adverse events.

Data synthesis, validation and analysis

Once the individual participant data from the primary studies have been merged in the common dataset, descriptive analyses will be conducted to identify data outliers, missing data, and unexpected inconsistencies. The project coordinator will prepare data clarification reports and will communicate with the principal investigators or their delegates to resolve these queries. Next, we plan to conduct preliminary analyses aimed at replicating the findings of the individual published studies, to validate the centralized database and data importation. Once the IPDMA team is satisfied with the merged dataset, the database will be locked and the planned analyses for the IPDMA synthesis will be conducted.

The individual patient data will be analyzed in a similar manner to an RCT, however, the analysis will account for clustering at the study level. The primary analysis will include all women who are eligible for AFFIRM and will examine the risk of the primary composite outcome in the treatment (LMWH) and control arms based on intention-to-treat. Secondary univariate analyses will be done for each of the pregnancy complications included in the composite outcome. On-treatment sensitivity analyses will be conducted for the primary and secondary outcomes.

Subgroup analyses

We have planned several subgroup analyses; these were selected because they are clinically plausible and there is evidence that they may be relevant. If certain subgroups are found to be small (≤5 subjects) we will merge subgroups as appropriate.

Women will be analyzed in subgroups according to the previous pregnancy complications that were experienced. Prior pre-eclampsia subgroups are any pre-eclampsia, severe pre-eclampsia, early-onset pre-eclampsia, and severe or early onset pre-eclampsia. Subgroups according to prior SGA are SGA <10th percentile, SGA <5th percentile, SGA <3rd percentile, prior pre-eclampsia and SGA <10th percentile, prior pre-eclampsia and SGA <5th percentile, prior pre-eclampsia and SGA <3rd percentile. Subgroups of women with prior placental abruption are any placental abruption, placental abruption leading to delivery <37 weeks' gestation, placental abruption leading to delivery < 34 weeks' gestation, and placental abruption with pre-eclampsia. Participants will be grouped for analysis according to the gestational age of prior pregnancy loss: >12 weeks' gestation, >16 weeks' gestation, and >20 weeks' gestation. Demographic subgroups are according to maternal age (<35 years or ≥35 years) and ethnic group (Caucasian, Black, Asian or other).

Women will be grouped according to personal characteristics and risk factors. For thrombophilia the subgroups are women with weak thrombophilia (Factor V Leiden [FVL] or prothrombin gene mutation [PGM]); moderate thrombophilia (protein C deficiency, protein S deficiency); strong thrombophilia (antithrombin deficiency, antiphospholipid antibodies, combined thrombophilia ≥1 type, homozygous FVL or PGM); or no thrombophilia. Participants will be grouped according to personal history of venous thromboembolism (VTE), family history of VTE, and no VTE history.

Quality assessment will be conducted for all eligible studies using the tool for assessing risk of bias from the Cochrane Handbook for reviews of interventions43 and reported on a study level. These assessments will also be used to inform subgroup analyses and sensitivity analyses to explore whether these biases may have affected the IPDMA analysis. We plan to examine the randomization integrity once the data from the original trials have been combined. We will endeavour to compare the original randomization lists with actual randomization to test the integrity of the allocation concealment. We will also compare the baseline characteristics of participants who have been randomized to the LMWH and no LMWH groups at the study level and aggregate level to see if there are imbalances between the groups that may suggest a lack of integrity in randomization processes.

Knowledge translation

Once the results of the analyses are available, they will be circulated to all investigators and collaborators and a teleconference will be scheduled to discuss the findings and their interpretation. Regardless of the IPDMA results, they will be disseminated. Dr Shannon Bates is the principal knowledge user for this project. She will provide input throughout the project and will be a leader for the knowledge translation phase of the study. The principal investigators of the identified eligible RCTs (Drs Rey, Martinelli, de Vries, Gris, Rodger, Middeldorp, Schleussner, and Kaaja) are all experienced researchers and also practicing

physicians who are knowledge users. Furthermore, these team members are all involved in leadership roles in their institutions and countries, including practice guideline development, and have the potential to considerably influence the international community of healthcare providers in a variety of settings.

The strategies for knowledge translation will rely heavily on the input from all involved knowledge users and will take into consideration the suitability of proposed media and/or approach for different practice settings and international contexts. Traditional methods, such as publication in a peer-reviewed journal, geared towards either a generalist or specialist audience, will be employed. Results will also be presented at international meetings; it is anticipated that knowledge users (clinicians) in hematology, obstetrics, and family medicine will be targeted. In addition, patient advocacy and education groups (such as the Pre-eclampsia Foundation, the North American Thrombosis Forum, and Thrombosis Canada) will be provided with the results in a language and format suitable to a non-medical audience.

Discussion

This IPDMA will permit the investigators to explore which women within the heterogeneous group of patients with placenta-mediated complications benefit and which women do not benefit from low-molecular-weight heparin injections throughout pregnancy.

Ethics, privacy and security
The subjects in each of the RCTs all provided informed consent to participate in the original trial. We will not be seeking individual consent for the secondary use of the data for the following reasons: the objectives of the IPDMA are consistent with the original trials, there are no risks or benefits associated with this analysis, no identifying information will be transferred, and it would be logistically time consuming and, in some cases, impossible to contact the women who participated. In order to ensure patient confidentiality any identifying information will be removed from the original dataset before it is transferred. The IronKey™ flash drive includes numerous security features including hardware-based encryption, a random password generator, two-factor authentication, and a self-destruct mechanism which make it extremely unlikely that the dataset can be accessed by anyone other than the intended recipient. Once the data are merged in Ottawa in the common database, they will be stored on the research institute's network which has multiple security features and regular backup procedures in place.

Limitations and challenges
One relevant potential drawback of IPDMA is biased pooling of data. Bias can be introduced when eligible studies are missed, when authors do not provide their data for the analysis, when the outcomes are different across studies, and when outcome and covariate data are missing from included studies.[31] Our recently completed pooled summary meta-analysis was

a successful collaboration of five principal investigators.1 In addition to the team members from these five trials, the principal investigators of four additional trials have committed to provide data for the AFFIRM meta-analysis. These are the largest and most robust trials completed in this area.

The multinational research team has representation from Canada, the Netherlands, France, Italy, Germany, and Finland. Almost all review team members attended the face-to-face IPDMA planning meeting. To protect against the misclassification of outcomes, the AFFIRM review team discussed each outcome at this meeting until consensus on detailed definitions and diagnostic criteria was reached. Definitions for all variables to be included in the IPDMA common dataset are documented in a data dictionary that was reviewed, revised according to team feedback, and finalized. Despite this, we recognize that challenges will be encountered due to variability in how the variables were originally defined and collected in each of the nine trials. In some cases it will be necessary to consult the original clinical records to obtain complete information for the IPDMA which will be a labor-intensive process. Another challenge is the diversity in language of the original datasets (English, French, Dutch, Italian, and German) that will necessitate translation when the data are recoded. Attention to detail, careful documentation, and excellent communication will be instrumental to the successful completion of this IPDMA.

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5

Low-molecular-weight heparin does not prevent recurrent placenta-mediated pregnancy complications: an individual patient data meta-analysis of randomized controlled trials

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Abstract

Background: Placenta-mediated pregnancy complications include pre-eclampsia, late pregnancy loss, placental abruption, and birth of a small-for-gestational-age (SGA) neonate. These complications are leading causes of maternal, fetal, and neonatal morbidity and mortality in high-income countries. Affected women are at high risk of recurrence in subsequent pregnancies; however, effective strategies to prevent recurrence are absent. Findings from our previous study-level meta-analysis suggested that low-molecular-weight heparin reduced the risk of recurrent placenta mediated pregnancy complications. However, we identified significant heterogeneity in the results, possibly due to trial design or inclusion criteria. To identify which patients benefit from, and which outcomes are prevented by, low-molecular-weight heparin, we did an individual patient data meta-analysis.

Methods: We did a systematic review in May, 2013, which identified eight eligible randomised trials done between 2000 and 2013 of low-molecular-weight heparin to prevent recurrent placenta-mediated pregnancy complications. We excluded studies on the basis of the wrong population, the study being ongoing, inability to confirm eligibility of participants, intervention stopped too early, and no response from the principal investigator. We requested individual patient data from the study authors for eligible women (women pregnant at the time of the study with a history of previous pregnancy that had been complicated by one or more of the following: pre-eclampsia, placental abruption, birth of an SGA neonate [<10th percentile], pregnancy loss after 16 weeks' gestation, or two losses after 12 weeks' gestation) and recoded, combined, and analysed the data for our meta-analysis. The primary outcome was a composite of early-onset (<34 weeks) or severe pre-eclampsia, birth of an SGA neonate (<5th percentile), late pregnancy loss (≥20 weeks' gestation), or placental abruption leading to delivery, assessed on an intention-to-treat basis. We assessed risk of bias with the Cochrane Risk of Bias tool. This study is registered with PROSPERO, number CRD42013006249.

Findings: We analysed data from 963 eligible women in eight trials: 480 randomly assigned to low-molecular-weight heparin and 483 randomly assigned to no low-molecular-weight heparin. Overall, the risk of bias was not substantial enough to affect decisions regarding trial inclusion. Participants were mostly white (795/905; 88%) with a mean age of 30·9 years (SD 5·0) and 403/963 (42%) had thrombophilia. In the primary analysis, low-molecular-weight heparin did not significantly reduce the risk of recurrent placenta-mediated pregnancy complications (low-molecular-weight heparin 62/444 [14%] versus no low-molecular-weight heparin 95/443 (22%) absolute difference −8%, 95% CI −17·3 to 1·4, p=0·09; relative risk 0·64, 95% CI 0·36–1·11, p=0·11). We noted significant heterogeneity between single-centre and multicentre trials. In subgroup analyses, low-molecular-weight heparin in multicentre trials reduced the primary outcome in women with previous abruption (p=0·006) but not in any of the other subgroups of previous complications.

Interpretation: Low-molecular-weight heparin does not seem to reduce the risk of recurrent placenta-mediated pregnancy complications in at-risk women. However, some decreases in event rates might have been too small for the power of our study to explore.

Introduction

Placenta-mediated pregnancy complications, including pre-eclampsia, birth of a small-for-gestational-age (SGA) neonate, placental abruption, or late pregnancy loss are common and lead to substantial maternal and fetal or neonatal morbidity and mortality.[1-3] The risk of recurrent placenta-mediated pregnancy complications in subsequent pregnancies is important,4-6 and these complications may be multiple (for example both pre-eclampsia and SGA) and not solely a repeat of the placenta-mediated complication experienced in a previous pregnancy.[4-6]

No highly effective preventive strategies for use in subsequent pregnancies exist. Aspirin offers small risk reductions in patients with previous pre-eclampsia and SGA; however, it might be more effective at reducing risk (providing around 40% relative risk reduction) if started before 16 weeks' gestation.[7;8] There are no proven preventive strategies for the other complications. The cause(s) of placenta-mediated pregnancy complications remain controversial and are likely to be multifactorial. However, placental microvascular and macrovascular thrombosis is a frequent, overlapping, pathophysiological link in many pregnancies affected by placenta-mediated complications,[3] and anticoagulants could prevent recurrence of these complications.

Low-molecular-weight heparin is the anticoagulant of choice in pregnancy because it does not cross the placenta and has a favourable maternal safety profile with low risk of major bleeding, heparin-induced thrombocytopenia, or heparin-induced osteoporosis.[9] Nonetheless, low-molecular-weight heparin must be administered by burdensome daily or twice-daily subcutaneous injections, is costly, and might complicate regional anaesthetic options if not discontinued within 12–24 h of labour onset. Low-molecular-weight heparin might also play other roles, including promotion of placental angiogenesis during the first and second trimesters of pregnancy and promotion of soluble vascular endothelial growth factor receptor-1 expression during the first trimester[10;11] that could also contribute to a reduced risk of placenta-mediated pregnancy complications.

Findings from some randomised controlled trials of whether low-molecular-weight heparin can prevent recurrent placenta-mediated pregnancy complications suggest an important treatment effect[12-16] but this finding has not been universal.[17-22] We previously published a pooled summary-based study-level meta-analysis,[23] the findings from which strongly suggested that low-molecular-weight heparin reduces the risk of placenta-mediated complications in subsequent pregnancies (relative risk reduction 0·52, 95% CI 0·32–0·86). However, this meta-analysis, with aggregate data, was limited by substantial statistical (I^2 69%) and clinical heterogeneity. Results from single-centre trials, and trials that recruited women with severe previous placenta-mediated complications, showed a beneficial effect of low-molecular- weight heparin, raising the possibility that either single-centre bias was driving the summary effects, or that low-molecular-weight heparin was not effective in women with previous non-severe placenta-mediated pregnancy complications.[23] Additionally, many of the component studies recruited women with heterogeneous previous placenta-mediated pregnancy complications, and explored effects of low-molecular-weight heparin

on composite outcomes that included a mix of these complications. These uncertainties lead to the questions of whether low-molecular-weight heparin is effective at all, whether it is only beneficial in subgroups of women with previous severe placenta-mediated pregnancy complications, and whether low-molecular-weight heparin prevents all or only some of these complications.

The trials of low-molecular-weight heparin to prevent placenta-mediated pregnancy complications were all academically sponsored and took many years to complete. To await results from future individual trials to address these questions would leave many patients without clear guidance in the interim. Therefore we did an individual patient data meta-analysis to account for study-centre effects, and to explore the effect of low-molecular-weight heparin in subgroups of women with previous placenta-mediated complications and on individual outcomes.

Methods

Search strategy and selection criteria

We did a systematic review to identify potentially eligible trials for meta-analysis. Detailed review methods, including the search strategy, search results in a PRISMA flow diagram, and a description of the trials identified are described in the published protocol.[24] Randomised controlled trials that used a low-molecular-weight heparin intervention for the prevention of recurrent placenta-mediated pregnancy complications were eligible. We developed electronic search strategies and tested them through an iterative process by an experienced medical information specialist in consultation with the review team. In May, 2013, using the OVID platform, we searched OVID MEDLINE, OVID MEDLINE in-process and other non-indexed citations, and Embase classic (appendix). We also searched the Cochrane Library and ClinicalTrials.gov to identify relevant ongoing and completed trials. We used search terms such as "hypertension", "pregnancy induced", "placental insuffi ciency", "heparin", and "low-molecular-weight" and keywords such as "pre-eclampsia", "abruption", and "LMWH". Vocabulary and syntax were adjusted across databases. Animal studies were excluded but there were no language or date restrictions for any of the searches. We sought additional references through hand-searching the bibliographies of relevant items. The study population of interest included currently pregnant women who had previous pregnancies complicated by one or more of the following: pre-eclampsia, placental abruption, birth of an SGA neonate (less than the 10th percentile), pregnancy loss after 16 weeks' gestation, or two losses after 12 weeks' gestation.

Of the potentially eligible studies identified, we included eight trials in the primary and subgroup analyses, and excluded eight others for the following reasons: wrong population,[25;26] trial ongoing (EPPI, HEPEPE, HOPPE trials), inability to confirm eligibility of participants,[22] LMWH intervention stopped too early in pregnancy,[21] and no response from the principal investigator.[27] Additional details about included and excluded studies are available in the protocol.[24]

Data extraction

The lead investigators of eligible trials and statisticians who were familiar with the trial data met in person to reach consensus on the study outcomes and variables before data extraction. Detailed definitions and diagnostic criteria for all study outcomes are in the study protocol and we used a data dictionary that includes the definitions and coding for all individual patient data meta-analysis variables to enable standardisation across studies. We developed a Microsoft Excel 2010 template to ensure consistency of the anonymised and recoded individual patient data. Ethics approval was obtained for each included trial before data were recoded and combined for meta-analysis.

The primary outcome of our individual patient data meta-analysis was a composite outcome including four pregnancy complications: early-onset or severe preeclampsia, birth of an SGA neonate with a birthweight less than the 5th percentile, placental abruption, and late pregnancy loss. Early-onset pre-eclampsia was defined as being diagnosed at less than 34 weeks' gestation. Severe pre-eclampsia was characterised by at least one criterion indicative of severe disease, including systolic blood pressure at least 160 mm Hg or diastolic blood pressure at least 110 mm Hg, proteinuria of more than 0·5 g/24 h, raised liver enzymes (more than two times the local upper range of normal), platelets less than 100 × 10^{9}/L, pulmonary oedema, seizures (eclampsia), headache or other neurological manifestation (stroke, intracranial haemorrhage, cerebral oedema, hyper-reflexia, or visual impairment), coagulopathy, oliguria (<30 mL/h), or HELLP syndrome (haemolysis, elevated liver enzymes, and low platelet count). Birth of an SGA neonate with a birthweight less than the 5th percentile was determined using local sex-specific and gestational age-specific birthweight charts. Placental abruption required a clinical diagnosis of placental abruption leading to delivery. Late pregnancy loss was defined as occurring at or after 20 weeks' of gestation that could not be accounted for by other factors, including fetal chromosomal abnormalities, maternal infection, cervical insufficiency or incompetence, or an intentional termination of the pregnancy. We also did post-hoc analyses of data for birth of an SGA neonate with a birthweight less than the 3rd percentile. We had included birthweight less than the 10th and the 5th percentiles in the published protocol but neglected to include the more severe form of this outcome because of an oversight.

The data included participant characteristics (demographic characteristics, thrombophilia, and relevant medical history), pregnancy history and details of the current pregnancy and delivery, including infant data and pregnancy complications. Information about treatments during pregnancy, particularly related to low-molecular-weight heparin and aspirin, and associated adverse events were recorded. Analysis of osteoporotic fractures and maternal death were post-hoc because we had unintentionally not included these very rare events as secondary outcomes in our protocol.

Data synthesis and validation

Data from the original trials were recoded by local personnel who were familiar with the data. They populated the Excel template according to the criteria for each variable that had been agreed upon a priori by the group. eligibility of each participant was verified by the project

coordinator (NJL) before data were included in the common dataset. Participants who were lost to follow-up or who did not have adequate primary outcome data were excluded. The recoded data from eligible women were imported to SAS version 9.3 and data verification scripts were run by the coordinating statistician (RM) to identify inconsistencies, outliers, and illogical data. The project coordinator (NJL) prepared data clarification requests and sent them via email to the investigators and personnel who had done the recoding. Data lock of the common dataset and analyses were done after resolution of all clarification requests. The primary analyses of the original trials were replicated before the meta-analysis to ensure that the results from each trial could be reproduced.

Risk of bias assessments

Assessments of study quality for included trials were done independently by two investigators (ADM and NJL) according to the seven criteria in the Cochrane Risk of Bias tool.[28] Funding was added as an additional criterion. The criteria were graded as low risk, high risk, or unclear risk of bias, and all disagreements were resolved by consensus. When the information was not available in the published paper or a public registry, the trial's lead author was contacted by email to request clarification or additional information.

Data analysis

The primary analysis included all eligible women with outcome data, and examined the risk of the primary composite outcome in the treatment (low-molecular-weight heparin) and control arms based on intention-to-treat analysis. Secondary univariate analyses were done for each of the pregnancy complications included in the composite outcome and other pregnancy complications of different severity, as outlined in the analysis plan. We calculated risk differences using generalised estimating equations to adjust for clustering at the study level. If expected counts were less than five, an adjustment was considered unfeasible and no formal test was done. Subgroup analyses were planned a priori based on clinical plausibility and existing evidence that the subgroups might be relevant.[24] We used SAS version 9.3 for all statistical analyses. This study is registered with PROSPERO, number CRD42013006249.

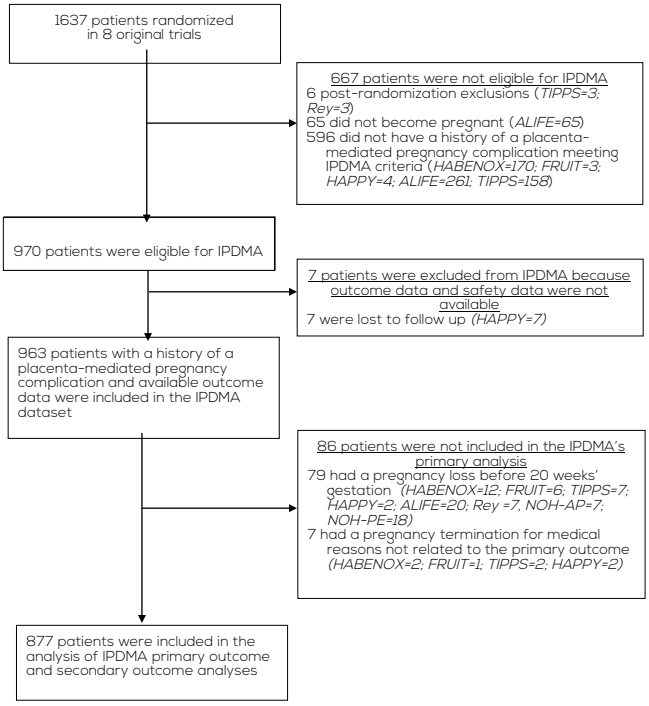
Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. MAR, TR, RM, and NJL had complete access to the data, and all authors had final responsibility for the decision to submit for publication.

Results

The dataset included a total of 963 eligible women from eight published trials that were done between 2000 and 2013 (figure). The ALIFE[20] and HABENOX[19] trials enrolled women with a history of pregnancy loss, NOH-PE[13] included women with previous pre-eclampsia and NOH-AP[16] enrolled those with prior placental abruption leading to delivery. HAPPY,[17]

Figure: Derivation of patients from the original studies



FRUIT,[12] TIPPS,[18] and the trial by Rey et al.[15] included women with a variety of prior placenta-mediated pregnancy complications, though pre-eclampsia was most common (Table 1). Trial participants may have experienced more than one previous complication.

Overall, the eight studies were very consistent in the risk of bias; full results of Study Quality Assessments are available as Supplementary Web Materials. All trials included open-label LMWH and, as such, blinding of patients was graded as high risk for six of the eight studies; the primary outcome of live birth was considered to be objective and unlikely to be influenced by lack of blinding for the other two studies.[18,19] Two studies were graded as 'unclear risk' for selective outcome reporting because trial registration or a protocol were not available[12,16] All studies had funding but the involvement of the supporting agency was clearly described in all papers as not influencing the results. Overall, the risk of bias was not substantial enough to impact trial inclusion decisions.

We noted no important imbalances between the treatment groups for demographic and clinical characteristics (table 2) or previous pregnancy history (table 3). The mean age of participants was 30·9 years (SD 5·0) and most were white. Most were enrolled in Europe (712/963; 74%), followed by North America (206/963; 21%) and Australia (45/963; 5%). Around a fifth had chronic hypertension and 8% (74/912) smoked. By design, all participants had had a previous pregnancy and most were in their second pregnancy. The most frequent previous placenta-mediated complication was preeclampsia and many women had severe or early-onset disease. About a third had given birth to an SGA neonate in less than the

Table 1: Trials included in the individual patient data meta-analysis (IPDMA)

Trial name publication year	Trial enrollment	Participants randomized in original trial	Participants eligible for IPDMA by qualifying prior complications*	LMWH intervention & control
TIPPS[18] 2014	Multi-national: 21 sites in Canada, USA Australia, the United Kingdom	N= 292 Thrombophilia and previous high risk criteria	N=113 Pre-eclampsia: 48 SGA: 47 Abruption: 18 ≥ 2 losses after 12 weeks GA: 36 ≥ 1 loss after 16 weeks GA: 62	Treatment: dalteparin 5000 IU to 20 weeks GA then 10,000 IU to 36 weeks GA Control: no dalteparin Aspirin use: permitted
FRUIT[12] 2012	Multi-national: 12 sites in the Netherlands, Sweden, Australia	N=139 Inheritable thrombophilia and prior early onset pre-eclampsia and/or SGA <10th percentile	N=136 Pre-eclampsia: 106 SGA: 47 Abruption: 11 ≥ 2 losses after 12 weeks GA: 41 ≥ 1 loss after 16 weeks GA: 43	Treatment: dalteparin 5000 IU + aspirin Control: aspirin alone
HAPPY[17] 2012	Multi-center: 8 sites in Italy	N=135 Prior pre-eclampsia, loss >15 weeks GA, SGA <10th percentile or placental abruption	N=124 Pre-eclampsia: 49 SGA: 53 Abruption: 20 ≥ 2 losses after 12 weeks GA: 41 ≥ 1 loss after 16 weeks GA: 41	Treatment: nadroparin 3800 IU Control: no nadroparin Aspirin use: discouraged
HABENOX [19] 2011	Multi-national: 4 sites in Finland, Sweden, the Netherlands	N= 207 Recurrent early or late miscarriage	N=37 Pre-eclampsia: 0 SGA: 1 Placental abruption: 4 ≥ 2 losses after 12 weeks GA: 14 ≥ 1 loss after 16 weeks GA: 29	Treatment 1: enoxaparin 40 mg + placebo aspirin Treatment 2: enoxaparin 40 mg + aspirin Control: aspirin alone
NOH-PE[13] 2011	Single center: France	N=224 Prior severe pre-eclampsia	N=224 Pre-eclampsia: 224 SGA: 58	Treatment: enoxaparin 4000 IU + aspirin Control: aspirin alone
NOH-AP[16] 2010	Single center: France	N=160 Prior placental abruption	N=160 Placental abruption: 160 Pre-eclampsia: 71 SGA: 44	Treatment: enoxaparin 4000 IU Control: no enoxaparin Aspirin use: if clinically indicated
ALIFE[20] 2010	Multi-center: 8 sites in the Netherlands	N=364 (299 pregnant) Recurrent pregnancy loss	N= 38 Pre-eclampsia: 4 SGA: 5 Placental abruption: 3 ≥ 2 losses after 12 weeks GA: 32 ≥ 1 loss after 16 weeks GA: 29	Treatment: nadroparin 2850 IU + aspirin Control 1: aspirin alone Control 2: placebo aspirin
Rey[15] 2009	Multi-center: 6 sites in Canada	N=116 Prior early pre-eclampsia, placental abruption, SGA<5th percentile, pregnancy loss >12 weeks GA	N=113 Pre-eclampsia: 93 SGA: 62 Placental abruption: 36 ≥ 2 losses after 12 weeks GA: 69 ≥ 1 loss after 16 weeks GA: 66	Treatment: dalteparin 5000 IU Control: no dalteparin Aspirin use: permitted

LMWH=low-molecular-weight heparin. SGA=small for gestational age. GA=gestational age. Loss=pregnancy loss.
*Participants might have had a history of more than one qualifying placenta-mediated pregnancy complication.

10th percentile of birthweight, and about a third had previous placental abruption. Preterm delivery before 34 weeks' gestation was also common, and 361/963 (37·5%) had had at least one previous pregnancy loss.

The prevalence of thrombophilia varied substantially by trial since in some cases this was stipulated by the protocol: the TIPPS[18] and FRUIT[12] trials required a diagnosis of thrombophilia for inclusion, whereas Rey et al.[15] excluded thrombophilic women. Overall, 41·6% of the IPDMA sample was diagnosed with thrombophilia. In the eight trials, women allocated to the LMWH treatment group received dalteparin, enoxaparin, or nadroparin; the drug, dosage and schedule of administration for each trial are shown in Table 1. The use of aspirin also differed by trial: in some it was provided to women in both the intervention and control groups,[12,13,19,20] in others the daily use of aspirin was at the discretion of the investigator and its use was recorded[15,18] or was given to women meeting specific clinical criteria,[16] and in one trial regular aspirin use was discouraged.[17] Two trials included a placebo control, matching the aspirin intervention.[19,20] The two trials that enrolled women with a history of pregnancy loss started the intervention very early, before seven weeks' of gestation,[19,20] most other trials required randomization before 12 weeks' gestation, while two allowed randomization to occur later,

Table 2: Characteristics of study participants

Characteristic	All Participants (N=963)	LMWH (n=480)	No LMWH (n=483)
Maternal age (mean, SD)			
(missing = 1)	30·9 (5·0)	30·9 (4·8)	30·8 (5·1)
Race (n, %)			
Caucasian	795/905 (87·9%)	409/457 (89·5%)	386/448 (86·2%)
Black	58/905 (6·4%)	23/457 (5·0%)	35/448 (7·8%)
Asian	31/905 (3·4%)	16/457 (3·5%)	15/448 (3·4%)
Other	21/905 (2·3%)	9/457 (2·0%)	12/448 (2·7%)
BMI median (lowest quartiles, highest quartile)			
(missing = 38)	25·0 (22·4- 27·8)	25·1 (22·5- 27·7)	24·9 (22·3-28·0)
Thrombophilia (n, %)			
FVL mutation, heterozygous	187/951(19·7%)	94/475 (19·8%)	93/476 (19·5%)
FVL mutation, homozygous	5/951 (0·5%)	2/475 (0·4%)	3/476 (0·6%)
Prothrombin mutation, heterozygous	78/943 (8·3%)	43/470 (9·2%)	35/473 (7·4%)
Prothrombin mutation, homozygous	1/943 (0·1%)	0/470 (0%)	1/473 (0·2%)
Antithrombin deficiency	6/939 (0·6%)	2/471 (0·4%)	4/468 (0·9%)
Protein C deficiency	18/945 (1·9%)	8/476 (1·7%)	10/469 (2·1%)
Protein S deficiency	106/943 (11·2%)	52/475 (11·0%)	54/468 (11·5%)
Antiphospholipid antibodies	31/882 (3·5%)	20/436 (4·6%)	11/446 (2·5%)
Smoker (n, %)	74/912 (8·1%)	36/453 (8·0%)	38/459 (8·3%)
Chronic hypertension (n, %)	154/757 (20·3%)	80/378 (21·2%)	74/379 (19·5%)
Diabetes (n, %)	0/832 (0%)	0/415 (0%)	0/417 (0%)
Venous thromboembolism (n, %)			
Maternal history	10/958 (1·0%)	5/478 (1·1%)	5/480 (1·0%)
Family history	34/840 (4·1%)	19/418 (4·6%)	15/422 (3·6%)
Arterial vascular disease (n, %)			
Family history	152/643 (23·6%)	84/320 (26·3%)	68/323 (21·1%)

Data are mean (SD), n/N (%), or median (lowest quartile and highest quartile). LMWH=low-molecular-weight heparin. FVL=Factor V Leiden.

but before 17 weeks'[15] or 20 weeks' of gestation.[18] All trials continued the intervention until at least 36 weeks' gestation or, in some cases, the onset of labor. Subgroup analyses allowed us to explore differences in participants and interventions.

In our primary outcome analysis, low-molecular-weight heparin did not significantly reduce the risk of recurrent placenta-mediated pregnancy complications (low-molecular-weight heparin 62/444 (14%) versus no low-molecular-weight heparin 95/443 (22%), absolute difference −8%, 95% CI −17·3 to 1·4, p=0·09; relative risk (RR) 0·64, 95% CI 0·36–1·11; p=0·11). We noted signify cant heterogeneity between single-centre and multicentre trials (table 4). In multicentre trials, no effect of low-molecular-weight heparin was shown in the primary composite outcome, its component outcomes, and almost all secondary outcomes. However, in single-centre trials, low-molecular- weight heparin seemed to prevent the composite primary outcome, the individual components of the composite outcome, and almost all secondary outcomes.

In subgroup analyses, we noted similar heterogeneity between multicentre and single-centre studies. In the multicentre trials, low-molecular-weight heparin did not prevent the composite primary outcome in women with previous pre-eclampsia, previous pregnancy loss, or previous

Table 3: Pregnancy history of study participants

Pregnancy History	All Participants (N=963)	LMWH (n=480)	No LMWH (n=483)
Gravida			
2	633/963 (65·7%)	318/480 (66·3%)	315/483 (65·2%)
≥3	330/963 (34·3%)	162/480 (33·8%)	168/483 (34·8%)
Previous live births			
0	147/963 (15·3%)	81/480 (16·9%)	66/483 (13·7%)
1	724/963 (75·2%)	355/480 (74·0%)	369/483 (76·4%)
2	66/963 (6·9%)	30/480 (6·3%)	36/483 (7·5%)
≥3	26/963 (2·7%)	14/480 (2·9%)	12/483 (2·5%)
Previous pregnancy losses			
0	602/963 (62·5%)	304/480 (63·3%)	298/483 (61·7%)
1	163/963 (16·9%)	79/480 (16·5%)	84/483 (17·4%)
2	72/963 (7·5%)	36/480 (7·5%)	36/483 (7·5%)
≥3	126/963 (13·1%)	61/480 (12·7%)	65/483 (13·5%)
Previous late pregnancy losses			
After 12 weeks GA (2 or more losses)	233/919 (25·4%)	114/461 (24·7%)	119/458 (26·0%)
After 16 weeks GA (1 or more losses)	270/930 (29·0%)	136/466 (29·2%)	134/464 (28·9%)
After 20 weeks GA (1 or more losses)	177/903 (19·6%)	90/457 (19·7%)	87/446 (19·5%)
Previous small for gestational age newborns			
SGA < 10th percentile	317/906 (35·0%)	161/453 (35·5%)	156/453 (34·4%)
SGA < 5th percentile	166/793 (20·9%)	82/403 (20·4%)	84/390 (21·5%)
SGA < 3rd percentile	70/680 (10·3%)	31/346 (9·0%)	39/334 (11·7%)
Previous placental abruption	286/886 (32·3%)	143/441 (32·4%)	143/445 (32·1%)
Previous preeclampsia			
Preeclampsia	595/963 (61·8%)	293/480 (61·0%)	302/483 (62·5%)
Severe preeclampsia	441/851 (51·8%)	225/434 (51·8%)	216/417 (51·8%)
Early-onset preeclampsia	307/801 (38·3%)	160/407 (39·3%)	147/394 (37·3%)
Previous preterm delivery			
< 37 weeks GA	751/960 (78·2%)	378/480 (78·8%)	373/480 (77·7%)
< 34 weeks GA	605/960 (63·0%)	307/480 (64·0%)	298/480 (62·1%)

Data are n/N (%). LMWH=low-molecular-weight heparin. GA=gestational age. SGA=small for gestational age. *Seven study participants had multiple gestations (twins) in the current pregnancy

birth of SGA neonates, irrespective of the severity of these previous complications (table 5). However, in single-centre trials, we noted a beneficial effect of low-molecular- weight heparin in women with previous preeclampsia, pregnancy loss, and previous birth of an SGA child, regardless of the severity of any previous complications. A beneficial effect of low-molecular-weight heparin was noted in women with previous placental abruption in both single-centre and multicentre trials.

In women with inherited or acquired thrombophilia and previous placenta-mediated pregnancy complications we noted no differences between the low-molecular- weight heparin groups and the groups allocated to receive no low-molecular-weight heparin in multicentre trials; however, we noted a beneficial low-molecular-weight heparin effect in women with inherited or acquired thrombophilia and previous placenta-mediated pregnancy complications in single-centre trials (table 5). This finding was replicated when subgroups of women with weak thrombophilia (ie, heterozygosity for the Factor V Leiden or prothrombin gene variants), moderate, and more potent thrombophilias were analysed separately. Exploration of differences in treatment dose, timing of low-molecular-weight heparin initiation, and concomitant aspirin use subgroups revealed a similar pattern of no benefit of low-molecular-weight heparin in multicentre trials but suggestion of a low-molecular-weight heparin benefit in single-centre trials (table 5).

In the analysis of safety outcomes, we noted few events and no differences between groups. We saw no serious adverse reactions to low-molecular-weight heparin, including heparin-induced thrombocytopenia, osteoporotic fractures, or maternal death. Ten allergic reactions occurred that were severe enough to require discontinuation of low-molecular-weight heparin; one was a control group crossover to low-molecular-weight heparin. In the antepartum period, four women haemorrhaged and met our definition of major bleeding. All of these events were attributable to placental abruption and are captured as primary outcome events. Two of these women were randomly assigned to low-molecular-weight heparin; the other two were in the control group and did not receive either low-molecular-weight heparin or aspirin. In the peripartum and postpartum periods, the incidence of major bleeding did not differ between the treatment and control groups.

Discussion

In this individual patient data meta-analysis, low-molecular-weight heparin did not significantly reduce the risk of recurrent placenta-mediated pregnancy complications in women with previous complications. Importantly, this finding also applies to subgroups of women with previous pre-eclampsia, previous severe pre-eclampsia, previous early-onset pre-eclampsia, previous late pregnancy loss (one or more losses after 16 weeks), previous recurrent late pregnancy loss (two or more losses after 12 weeks), previous births of babies who were mildly SGA (<10th percentile) or more severely SGA (<5th percentile).

The absence of effect of low-molecular-weight heparin might reflect the multifactorial pathophysiology for these placenta-mediated pregnancy complications. Indeed, the cumulative observational scientific literature exploring the association between thrombophilia and placenta-mediated pregnancy complications suggests a weak association with pregnancy loss, severe pre-eclampsia, SGA birth less than the 3rd percentile, and abruption, but no association with any pre-eclampsia or less severe SGA birth.[29;30] Overall, findings from observational research, and now experimental research, suggest that placental thrombosis might not be a major contributor to placenta-mediated pregnancy complications. As we learn more about the underlying disease mechanisms for placenta-mediated pregnancy complications and develop pragmatic diagnostic tools to identify when these mechanisms are in play, we might be able to define patient subgroups that could benefit from low-molecular-weight heparin. Results in a small subgroup of patients with previous abruption suggest low-molecular-weight heparin might prevent placenta-mediated pregnancy complications in subsequent pregnancies but this finding requires confirmation in future multicentre trials before it can be adopted in routine clinical practice. This finding might seem counter-intuitive, given that placental abruption is a bleeding complication. However, low-molecular-weight heparin might prevent the placental infarction that often precedes bleeding into placental infarcts, which manifests clinically as placental abruption. In the absence of strong evidence or proven treatment alternatives, personalised medicine and counselling will be important in the decision-making process when considering low-molecular-weight heparin for women

Table 4: Primary, secondary, and safety outcomes

	LMWH (n=480)	No LMWH (n=483)	Absolute difference (95% CI) p-value	LMWH (n=288)	No LMWH (n=291)	Absolute difference (95% CI) p-value	LMWH (n=192)	No LMWH (n=192)	Absolute difference (95% CI) p-value
Primary composite outcome of early-onset or severe pre-eclampsia, or SGA <5th percentile, or placental abruption, or pregnancy loss ≥ 20 weeks' gestation*	62/444 (14%)	95/433 (22%)	−8·0% (95% CI −17·3 to 1·4), p=0·09	47/263 (18%)	47/255 (18%)	−0·6% (95% CI −10·4 to 9·2), p=0·91	15/181 (8%)	48/178 (27%)	−18·7% (95% CI −21·6 to −15·7), p<0·0001
Secondary outcomes									
Placental abruption	15/469 (3%)	31/474 (7%)	−3·3% (95% CI − 6·7 to −0·1), p=0·0491	5/277 (2%)	7/282 (2%)	−0·7% (95% CI − 4·0 to 2·6), p=0·69	10/192 (5%)	24/192 (13%)	−7·3% (95% CI − 9·0 to −5·6), p<0·0001
Placental abruption leading to delivery	5/469 (1%)	10/474 (2%)	−1·0% (95% CI − 2·4 to 0·3), p=0·14	3/277 (1%)	5/282 (2%)	†	2/192 (1%)	5/192 (3%)	†
Any pregnancy loss*	46/477 (10%)	64/478 (13%)	−3·8% (95% CI − 9·5 to 2·0), p=0·20	30/285 (11%)	37/286 (13%)	−2·4% (95% CI − 11·3 to 6·5), p=0·60	16/192 (8%)	27/192 (14%)	−5·7% (95% CI − 7·8 to −3·7), p<0·0001
Pre-eclampsia§	41/444 (9%)	67/433 (15%)	−6·2% (95% CI − 13·1 to 0·6), p=0·08	29/263 (11%)	32/255 (13%)	−1·5% (95% CI − 10·0 to 7·0), p=0·73	12/181 (7%)	35/178 (20%)	−13·0% (95% CI − 16·4 to −9·6), p<0·0001
Severe pre-eclampsia§	22/442 (5%)	43/433 (10%)	−5·0% (95% CI − 11·2 to 1·3), p=0·12	19/261 (7%)	19/255 (7%)	−0·2% (95% CI − 6·4 to 6·0), p=0·96	3/181 (2%)	24/178 (13%)	−11·8% (95% CI − 16·6 to −7·1), p<0·0001
Early-onset pre-eclampsia§	18/444 (4%)	32/433 (7%)	−3·3% (95% CI − 7·9 to 1·2), p=0·15	11/263 (4%)	14/255 (5%)	−1·3% (95% CI −7·5 to 4·9 (p=0·68)	7/181 (4%)	18/178 (10%)	−6·2% (95% CI − 10·5 to −2·0), p=0·0037
Severe or early-onset pre-eclampsia§	31/444 (7%)	51/433 (12%)	−4·8% (95% CI − 11·6 to 2·0), p=0·17	24/263 (9%)	22/255 (9%)	0·5% (95% CI − 6·8 to 7·8), p=0·89	7/181 (4%)	29/178 (16%)	−12·4% (95% CI − 16·5 to −8·4), p<0·0001
HELLP syndrome§	2/384 (1%)	11/370 (3%)	−2·5% (95% CI − 4·4 to −0·6)(p=0·0112)	1/203 (<1%)	3/192 (2%)	†	1/181 (1%)	8/178 (4%)	†
SGA <10th percentile§	61/444 (14%)	94/429 (22%)	−8·2% (95% CI − 14·3 to −2·0), p=0·0094	47/263 (18%)	53/251 (21%)	−3·2% (95% CI − 9·6 to 3·1), p=0·32	14/181 (8%)	41/178 (23%)	−15·3% (95% CI − 19·1 to −11·5), p<0·0001
SGA <5th percentile§	27/443 (6%)	38/429 (9%)	−2·8% (95% CI − 5·4 to −0·1), p=0·0417	22/262 (8%)	23/251 (9%)	−0·8% (95% CI − 3·7 to 0·2), p=0·61	5/181 (3%)	15/178 (8%)	−5·7% (95% CI − 6·1 to −5·2), p<0·0001
SGA <3rd percentile§	13/443 (3%)	12/429 (3%)	−0·1% (95% CI − 1·9 to 2·2, p=0·89	13/262 (5%)	9/251 (4%)	1·4% (95% CI −1·3 to 4·1), p=0·32	0/181	3/178 (2%)	†
Pregnancy loss ≥20 weeks' gestation§	13/444 (3%)	18/432 (4%)	−1·2% (95% CI − 4·2 to 1·8), p=0·42	8/263 (3%)	5/254 (2%)	1·1% (95% CI − 2·1 to 4·2), p=0·50	5/181 (3%)	13/178 (7%)	−4·5% (95% CI − 7·0 to −2·1), p=0·0003
Preterm delivery <37 weeks' gestation§	131/431 (30%)	136/414 (33%)	−2·5% (95% CI −9·7 to 4·5), p=0·49	58/255 (23%)	48/249 (19%)	3·5% (95% CI − 1·3 to 8·2), p=0·15	73/176 (41%)	88/165 (53%)	−11·9% (95% CI − 13·5 to −10·3), p<0·0001
Preterm delivery <34 weeks' gestation§	28/431 (6%)	45/414 (11%)	−4·4% (95% CI − 9·0 to 0·3), p=0·07	17/255 (7%)	19/249 (8%)	−1·0% (95% CI −4·7 to 2·8), p=0·61	11/176 (6%)	26/165 (16%)	−1·0% (95% CI − 14·6 to −4·4), p=0·0003
Neonatal death within 28 days of birth§	3/423 (1%)	9/406 (2%)	−1·5% (95% CI − 3·1 to 0·1), p=0·07	1/247 (<1%)	2/241 (1%)	†	2/176 (1%)	7/165 (4%)	†
Safety outcomes									
Venous thromboembolism	1/468 (<1%)	2/457 (<1%)	†	1/276 (<1%)	2/265 (1%)	†	0/192	0/192	..
Allergic reaction to LMWH	9/480 (2%)	1/483 (<1%)	†	9/288 (3%)	1/291 (<1%)	†	0/192	0/192	..
Antepartum major bleeding‡	1/470 (<1%)	3/473 (1%)	†	0/278 (<1%)	0/281	..	1/192 (1%)	3/192 (2%)	†

Peripartum major bleeding	10/404 (2%)	12/395 (3%)	−0·3% (95% CI −1·6 to 1·0), p=0·30	10/212 (5%)	9/203 (5%)	0·2% (95% CI −2·0 to 2·6), p=0·80	0/192	2/192 (1%)	−1·0% (95% CI −2·5 to 0·4), p=0·50
Post-partum major bleeding	3/470 (1%)	4/473 (1%)	†	3/278 (1%)	4/281 (1%)	†	0/192	0/192	..
Thrombocytopenia	14/469 (3%)	6/476 (1%)	1·7% (95% CI −2·2 to 5·7), p=0·40	14/277 (5%)	6/284 (2%)	2·9% (95% CI −3·8 to 9·7, p=0·40	0/192	0/192	..
Heparin-induced thrombocytopenia	0	0	..	0	0	..	0	0	..
Osteoporotic fracture	0	0	..	0	0	..	0	0	..
Maternal death	0	0	..	0	0	..	0	0	..

Outcomes noted for study participants according to treatment allocation (intention to treat). Relative risk for the primary outcome difference for all trials 0•64, 95% CI 0•36–1•11; p=0•11. Data are n/N (%). LMWH=low-molecular-weight heparin. SGA=small for gestational age. ••=not applicable. *Excludes eight women with terminations for medical reasons other than the primary outcome. †Expected counts were less than five, therefore an adjustment was considered unfeasible and no formal test was done. §Excludes 86 women that had a pregnancy loss before 20 weeks' gestation or had a pregnancy termination for medical reasons other than the primary outcome. ‡All antepartum major bleeding was associated with the primary outcome event of placental abruption.

Table 5: Primary outcome according to patient subgroup

	All Trials			Multicentre trials			Single-centre trials		
	LMWH (n=444)	No LMWH (n=433)	Absolute difference (95% CI) p-value	LMWH (n=288)	No LMWH (n=291)	Absolute difference (95% CI) p-value	LMWH (n=192)	No LMWH (n=192)	Absolute difference (95% CI) p-value
Previous pregnancy complication subgroups									
Any pre-eclampsia	37/276 (13%)	73/285 (26%)	−12·2% (95% CI −20·2 to −4·3), p=0·0026	26/139 (19%)	36/146 (25%)	−6·0% (95% CI −18·2 to 6·3), p=0·34	11/137 (8%)	37/139 (27%)	−18·6% (95% CI −22·2 to −15·0), p<0·0001
Severe pre-eclampsia	26/212 (12%)	50/203 (25%)	−12·4% (95% CI − 21·8 to −2·9), p=0·0104	18/94 (19%)	20/88 (23%)	−3·6% (95% CI −22·3 to 15·2), p=0·71	8/118 (7%)	30/115 (26%)	−19·3% (95% CI −25·4 to −13·2), p<0·0001
Early-onset pre-eclampsia	23/152 (15%)	36/141 (26%)	−10·4% (95% CI −22·2 to 1·4), p=0·08	22/103 (21%)	25/95 (26%)	−5·0% (95% CI −20·7 to 10·8), p=0·54	1/49 (2%)	11/46 (24%)	−21·9% (95% CI −27·5 to −16·2), p<0·0001
Severe or early-onset pre-eclampsia	33/239 (14%)	57/228 (25%)	−11·1% (95% CI −20·7 to −1·7), p=0·0207	25/121 (21%)	27/113 (24%)	−3·2% (95% CI −18·4 to 12·0), p=0·68	8/118 (7%)	30/115 (26%)	−19·3% (95% CI −25·4 to −13·2), p<0·0001
Any previous loss after 12 weeks' gestation	22/128 (17%)	19/114 (17%)	0·5% (95% CI −10·7 to 11·8), p=0·93	22/128 (17%)	19/114 (17%)	0·5% (95% CI −10·7 to 11·8), p=0·93	0	0	..
One or more late losses after 16 weeks' gestation	21/120 (18%)	19/109 (17%)	0·07% (95% CI −11·9 to 12·2), p=0·99	21/120 (18%)	19/109 (17%)	0·07% (95% CI −11·9 to 12·2), p=0·99	0	0	..
Two or more late losses after 12 weeks' gestation	4/22 (18%)	2/14 (14%)	*	4/22 (18%)	2/14 (14%)	*	0	0	..
SGA <10th percentile	24/152 (16%)	40/145 (28%)	−11·8% (95% CI −25·3 to 1·7), p=0·09	21/105 (20%)	22/95 (23%)	−3·2% (95% CI −16·8 to 10·5), p=0·65	3/47 (6%)	18/50 (36%)	−30·0% (95% CI −40·0 to −19·3), p<0·0001
SGA <5th percentile	9/77 (12%)	20/77 (26%)	−14·3% (95% CI −27·1 to −0·7), p=0·04	8/59 (14%)	13/56 (23%)	−10·0% (95% CI −26·5 to 7·2), p=0·26	1/18 (6%)	7/21 (33%)	*
SGA <3rd percentile	6/31 (19%)	11/35 (31%)	12·1% (95% CI −35·7 to 11·5), p=0·32	6/21 (29%)	6/25 (24%)	4·6% (95% CI −6·3 to 15·5), p=0·41	0/10	4/10 (40%)	*
Any pre-eclampsia and SGA <10th percentile	13/91 (14%)	30/97 (31%)	−16·6% (95% CI −28·5 to −4·8), p=0·0058	11/53 (21%)	16/55 (29%)	−8·3% (95% CI −26·5 to 9·9), p=0·37	2/38 (5%)	14/42 (33%)	−27·8% (95% CI −37·5 to −18·7), p<0·0001
Any pre-eclampsia and SGA <5th percentile	6/53 (11%)	13/50 (26%)	−14·7% (95% CI −31·1 to 1·7), p=0·08	5/35 (14%)	8/33 (24%)	−10·0% (95% CI −36·8 to 16·9), p=0·47	1/18 (6%)	5/17 (29%)	*
Any pre-eclampsia and SGA <3rd percentile	3/15 (20%)	4/15 (27%)	*	3/5 (60%)	1/7 (14%)	*	0/10	3/8 (38%)	*
Any placental abruption	11/138 (8%)	33/134 (25%)	−16·7% (95% CI −23·0 to −10·4), p<0·0001	3/48 (6%)	9/47 (19%)	−12·9% (95% CI −22·1 to −3·7), p=0·006	8/90 (9%)	24/87 (28%)	−18·7% (95% CI −24·7 to −12·7), p<0·0001
Placental abruption leading to delivery	10/122 (8%)	32/118 (27%)	−18·9% (95% CI −22·8 to −15·1), p<0·0001	3/45 (7%)	9/42 (21%)	−14·8% (95% CI −23·3 to −6·3), p=0·0007	7/77 (9%)	23/76 (30%)	−21·2% (95% CI −33·3 to −9·0), p<0·0001
Any placental abruption with any pre-eclampsia	5/65 (8%)	20/69 (29%)	−21·3% (95% CI −29·7 to −12·9), p<0·0001	1/19 (5%)	7/21 (33%)	*	4/46 (9%)	13/48 (27%)	−18·4% (95% CI −29·0 to −7·7), p=0·0007
Thrombophilia									
No thrombophilia	26/258 (10%)	58/246 (24%)	−13·5% (95% CI −18·1 to −8·9), p<0·0001	11/103 (11%)	19/94 (20%)	−9·5% (95% CI −22·0 to 2·9), p=0·13	15/155 (10%)	39/152 (26%)	−16·0% (95% CI −17·0 to −15·0), p<0·0001
Weak thrombophilia (heterozygous FVL or PGM)	21/112 (19%)	24/114 (21%)	−2·3% (95% CI −17·6 to 13·0), p=0·77	21/86 (24%)	17/91 (19%)	5·7% (95% CI −5·1 to 16·5), p=0·29	0/26	7/23 (30%)	−30·0% (95% CI −49·2 to −11·6), p=0·0029
Moderate thrombophilia (deficiency of protein C or S)	6/40 (15%)	9/53 (17%)	−2·0% (95% CI −13·8 to 9·9), p=0·74	6/40 (15%)	8/51 (16%)	−0·7% (95% CI −11·6 to 10·2), p=0·90	0	0	..
Strong thrombophilia (antithrombin deficiency, antiphospholipid antibodies, homozygous FVL or PGM, or more than one thrombophilia)	9/34 (26%)	4/20 (20%)	*	9/34 (26%)	3/19 (16%)	*	0	0	..
LMWH treatment									
Low dose (nadroparin 2850 IU or 3800 IU; enoxaparin 4000 IU; or dalteparin ≤5000 IU per day)	42/354 (12%)	95/433 (22%)	−10·1% (95% CI −18·3 to −1·9), p=0·016	27/173 (16%)	47/255 (18%)	−2·8% (95% CI −12·8 to 7·2), p=0·58	15/181 (8%)	48/178 (27%)	−18·7% (95% CI −21·6 to −15·7), p<0·0001
Intermediate dose (>5000 IU dalteparin per day)	20/90 (22%)	95/433 (22%)	0·28% (95% CI −6·5 to 7·1), p=0·93	20/90 (22%)	47/255 (18%)	3·8% (95% CI −2·7 to 10·3), p=0·25	..	..	..
Aspirin treatment									

Daily aspirin	37/260 (14%)	72/262 (27%)	−13·3% (95% CI − 23·2 to -3·3), p=0·0091	26/146 (18%)	32/140 (23%)	−5·1% (95% CI − 15·7 to 5·6), p=0·35	11/114 (10%)	40/122 (33%)	−23·1% (95% CI - 37·4 to -8·9), p=0·0014
No aspirin	25/181 (14%)	22/156 (14%)	−0·3% (95% CI − 9·0 to 8·4), p=0·95	21/114 (18%)	14/100 (14%)	4·4% (95% CI − 3·3 to 12·2), p=0·26	4/67 (6%)	8/56 (14%)	−8·3% (95% CI - 19·1 to 2·5), p=0·13
Time of LMWH initiation									
Before 10 weeks' gestation	38/303 (13%)	95/433 (22%)	−9·4% (95% CI - 19·1 to 0·3), p=0·06	23/122 (19%)	47/255 (18%)	0·4% (95% CI − 12·1 to 12·9), p=0·95	15/181 (8%)	48/178 (27%)	−18·7% (95% CI - 21·6 to -15·7), p<0·0001
Before 16 weeks' gestation	58/416 (14%)	95/433 (22%)	−8·0% (95% CI − 17·8 to 1·8), p=0·11	43/235 (18%)	47/255 (18%)	−0·1% (95% CI − 11·0 to 10·7), p=0·98	15/181 (8%)	48/178 (27%)	−18·7% (95% CI - 21·6 to -15·7), p<0·0001
Before 20 weeks' gestation	62/441 (14%)	95/443 (21%)	−7·9% (95% CI − 17·4 to 1·6), p=0·10	47/260 (18%)	47/255 (18%)	−0·4% (95% CI − 10·4 to 9.7), p=0·94	15/181 (8%)	48/178 (27%)	−18·7% (95% CI - 21·6 to -15·7), p<0·0001

Data are n/N (%). LMWH=low-molecular-weight heparin. SGA=small for gestational age. FVL=Factor V Leiden. PGM=prothrombin gene mutation. ••=not applicable . *Expected counts were less than five, therefore an adjustment was considered unfeasible and no formal test was done.

with a history of placental abruption.

Our previous pooled summary-based meta-analysis[23] of six trials included 848 pregnant women with a history of pre-eclampsia, birth of an SGA neonate (<10th percentile), placental abruption, or late pregnancy loss (after more than 12 weeks' gestation). The primary finding was that 67 of 358 (19%) women given low-molecular- weight heparin during pregnancy had recurrent severe placenta-mediated pregnancy complications, compared with 127 of 296 (43%) women with no low-molecular-weight heparin (RR reduction 48%, 95% CI 14–68%; I^2 69%). However, since these meta-analysis results applied to a heterogeneous group of women with a mixture of previous placenta-mediated pregnancy complications of varying severity, and the primary outcome for the meta-analysis was a composite of all placenta-mediated complications (also of varying severity), which subgroups of women derive the most benefit from low-molecular-weight heparin was unclear (ie, which outcomes were reduced and outcomes of what severity were affected). The limitations of this meta-analysis supported the need to do an individual patient data meta-analysis.

A strength of our study was the inclusion of individual patient data from the largest, and almost all, completed trials that assessed low-molecular-weight heparin to prevent placenta-mediated pregnancy complications. Limitations included that the primary analysis of the individual patient data meta-analysis also included a heterogeneous group of women with different previous placenta-mediated pregnancy complications, the interventions in the eight trials included three low-molecular- weight heparins of differing doses, gestational age varied at treatment initiation, co-intervention with aspirin varied, and that the primary outcome was a composite of four complications. However, the advantages of individual patient data meta-analyses lie in the ability to do subgroup analyses that are hypothesised to be clinically relevant, provision of a rich dataset from individual patient data, and greater statistical power than conventional meta-analyses.[31;32] The individual patient data meta-analysis enabled us to explore clinical, methodological, and statistical heterogeneity more robustly. We acknowledge that some of the subgroups included patients with rare outcomes and these analyses were restricted by small samples.

Other limitations of our study are that there might have been smaller absolute decreases in event rates than we had sufficient power to explore. However, this limitation depends strongly on what is valued as the minimal clinically important difference. Given our observed composite primary outcome event rate of 18% in the control group of the multicentre trials, an adequately powered (80%) trial to detect a 3%, 6%, or 9% absolute reduction (17%, 33%, or 50% RR reduction, or number needed to treat of 33, 17, or 11) would require 2400, 555, or 226 participants per group, respectively. Hence, if clinicians, patients, and policy makers are willing to accept high numbers needed to treat, and hence small minimal clinically important differences, then larger clinical trials will be required to definitively answer this question. However, we believe that most would agree that numbers needed to treat must be reasonably small (eg, ten or less) to justify using these burdensome and expensive injections throughout pregnancy. Finally, three ongoing trials (NCT00986765, NCT01388322, and ACTRN12609000699268) comparing low-molecular-weight heparin to no low-molecular-weight heparin in women with previous pre-eclampsia will provide additional data to explore

smaller absolute risk differences with improved power.

The results obtained in single-centre trials contrasted starkly with those from the multicentre trials. However, this effect has been observed in critical-care trials[33] and in many other disease areas.[34] Indeed, in a meta-epidemiological study, single-centre trials exaggerated treatment effects by more than 25%, and the investigators suggested that results from single-centre trials should be considered separately from those from multicentre trials when meta-analyses are interpreted. Possible explanations for differences in treatment effects in single-centre trials compared with multicentre trials exploring low-molecular- weight heparin to prevent recurrent placenta-mediated pregnancy complications include publication bias, lower trial quality, and co-interventions.

Publication bias might occur when findings from small, single-centre trials with negative results are not published and hence would not be included in our meta-analysis. Although we searched trial registration websites for any trials to avoid publication bias, clinical trial registration only became mandatory in many jurisdictions in the early 2000s, leading to the possibility that small trials with negative results were unpublished and never registered. Single-centre trials are sometimes of lower quality and empirically trials of lower quality are associated with larger treatment eff ects.[35;36] Indeed, our risk of bias assessment suggests that the single-centre trials in our individual patient data meta-analysis were at higher risk of bias because the single-centre trials were not registered. Finally, co-intervention, such as closer follow-up of women in the low-molecular-weight heparin arms in the single-centre trials, could have led to an apparent low-molecular weight heparin treatment effect. Closer follow-up, in and of itself, might prevent recurrent pregnancy loss.[37;38] We do not believe that the single-centre trials showed a greater treatment effect because of differences in treatment regimens, because the highest doses of low-molecular-weight heparin were used in a multicentre trial.

In conclusion, overall low-molecular-weight heparin does not seem to reduce the risk of recurrent placenta-mediated pregnancy complications. Results in a small subgroup of women with previous abruption suggest low-molecular-weight heparin might prevent placenta-mediated complications in this population, but this finding should be replicated in future multicentre trials.

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6

Aspirin adherence during high-risk pregnancies, a questionnaire study

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Abstract

Objective: Aspirin reduces the risk of recurrent hypertensive disorders of pregnancy (HD) and fetal growth restriction (FGR). This study examined the non-adherence rates of aspirin in women with high-risk pregnancies.

Study design: All consecutive women between 24 and 36 weeks gestation with an indication for aspirin use during pregnancy were invited for this study. A survey was used which included two validated questionnaires, the simplified medication adherence questionnaire (SMAQ) and the Beliefs and Behaviour Questionnaire (BBQ).

Main outcome measures: To determine the non-adherence rates of aspirin, and to identify the beliefs and behavior concerning aspirin.

Results: Indications for aspirin use during pregnancy were previous HD, FGR, intrauterine fetal death or current maternal disease. Non-adherence rates according to the SMAQ and BBQ were 46.3% and 21.4% respectively. No differences in demographic background or obstetrical characteristics between adherent and non-adherent women could be demonstrated.

Conclusions: Adherence for aspirin in this high-risk population cannot be taken for granted. The non-adherence rates in pregnant women are comparable with the non-adherence rates for aspirin in the non-pregnant population.

Introduction

Aspirin reduces the risk of (recurrent) hypertensive disorders of pregnancy (HD) and fetal growth restriction (FGR).[1,2] Besides women with previous HD or FGR, women with maternal diseases like chronic hypertension, diabetes, chronic kidney disease and autoimmune diseases like systemic lupus erythematosus and antiphospholipid syndrome are at elevated risk to develop HD.[3,4] The number of indications is increasing, also due to recommendations of the World Health Organization and the NICE guidelines in which women at increased risk for pregnancy complications are recommended to take aspirin during pregnancy.[5,6] Commencing aspirin early in pregnancy (<16 weeks gestation) appears to be more effective than start at later gestation,[1,7] although a recent meta-analysis does not support this finding.[8] The preferred moment of ingestion of aspirin is at bedtime.[9,10] The use of aspirin in pregnancy is considered safe since relatively little complications are associated with the use of aspirin, although long term follow-up of infants is limited.[11]

Strategies to prevent (recurrent) HD and FGR are limited, and aspirin is described to reduce the risk to develop HD and FGR by at least 10%.[1] Adherence to aspirin seems important to receive the most optimal effect. One could speculate that missing a few tablets of aspirin already has an impact on its working mechanism, since some patient groups have persistent uninhibited platelet activity when using aspirin once a day.[12]

We are not aware of any studies regarding adherence to aspirin in pregnancy. Adherence during pregnancy for other medicines has been studied, including iron supplements, anti-convulsants, anti-retrovirals, anti-diabetics and medicines for chronic conditions like cardiovascular diseases and ulcerative colitis.[13-16] A wide range of non-adherence rates was reported, varying from 20 to 80%.[13-16] Two studies described that adherence is equal or even higher during pregnancy compared to postpartum or non-pregnant patients.[14,15] On the other hand, another study reported lower adherence rates of medicines for chronic conditions during pregnancy compared to the general population.[16] A review examining aspirin non-adherence in non-pregnant patients reported non-adherence rates from approximately 10% to over 50%.[17] It is questionable whether these results also apply to pregnant women, since pregnant women could either be more adherent because of the clear motivation to prevent pregnancy complications and the short period of time the medication needs to be taken. Yet, women could be less adherent due to fear of possible harm for the fetus or side effects. Therefore the aim of this study was to investigate aspirin adherence and beliefs and behavior of pregnant women concerning aspirin. It is of importance to investigate adherence to aspirin, because of its increased use in the obstetric field, as it is one of the few methods to decrease the incidence of HD in pregnancy.

Methods

Study population

This observational study was conducted between February 2015 and February 2016. All

pregnant women of 18 years or older with an indication for aspirin use (acetylsalicylic acid 80 mg) during pregnancy were invited between 24 and 36 weeks gestation. Women who already had an indication for aspirin prior to their pregnancy were excluded (for example a history of cerebrovascular event), as well as participants who were not able to complete the survey in Dutch. Participants were recruited from the VU University Medical Center in Amsterdam, a tertiary university hospital in the Netherlands. The Institutional Review board of the VU University Medical Center in Amsterdam, the Netherlands, concluded that official approval from a medical ethical committee was not needed due to the character of this study. All women gave written informed consent.

Procedures

The main outcomes were non-adherence rates, beliefs and behavior regarding aspirin. A single survey was performed, which consisted of four parts, [59] questions in total.

1. Socio-demographic background and general history.
2. Pregnancy related questions including questions about prior and current pregnancies.
3. Validated simplified medication adherence questionnaire (SMAQ).[18] The six-item SMAQ is a tool to measure the level of self-reported adherence. It has a satisfactory internal consistency with a Cronbach's alpha of 0.75.[18] Questionnaires with a Cronbach's alfa > 0.70 are considered acceptable.19 A woman was considered to be non-adherent when either a positive response to any of the qualitative questions was given or more than two doses were missed over the past week or more than two days without medication occurred during the past three months
4. Validated Beliefs and Behaviour Questionnaire (BBQ).20 The BBQ measures three categories of questions: beliefs, experience and behavior to assess adherence on a five-point Likert scale.[20] Each category consists of two sub-scales (confidence-concerns, satisfaction-disappointment and adherence- non-adherence). The internal consistency (Cronbach's alfa's) of all the sub-scales is >0.70, except the subscale 'confidence', with a Cronbach's alfa of 0.62. A woman was considered to be non-adherent when low scores on both the subscale 'adherence' (score below [19]) and the subscale 'non-adherence' (score higher than 8) were reported. In both the SMAQ and the BBQ, some questions were modified to focus on aspirin use during pregnancy. For example 'medicine' was replaced by 'aspirin' or the term 'during pregnancy' was added.

Definitions

Participants were considered to be non-Caucasian when the participant or one of the parents was of non-European descent. Educational level was determined as highest level of education and categorized as follows: low (no education or primary school), middle (secondary or vocational education) and high (college or university). Indications for aspirin use during pregnancy were divided into four groups: HD, FGR, intrauterine fetal death (IUFD) and maternal diseases. HD included pregnancy induced hypertension (PIH), preeclampsia, eclampsia and HELLP-syndrome. Maternal diseases included chronic hypertension, systemic lupus erythematosus, antiphospholipid syndrome and nephropathy.

Statistical analyses

Baseline characteristics were examined using descriptive statistics. To compare characteristics between adherent and non-adherent women according to the SMAQ an independent t-test, Chi^2-test or Fishers exact test was used. When missing data occurred, this participant was not included in that specific analysis. A two-tailed *p*-value inferior to 0.05 was considered to be significant. Statistical analyses was performed with IBM SPSS 22 (SPSS Inc., Chicago, IL).

Results

Out of 53 women, two refused participation and nine women did not return the survey, resulting in 42 participants in this study (response rate 79.2%). Baseline characteristics are depicted in Table 1. Indications for aspirin were previous HD (52.4%), previous FGR (38.1%), previous IUFD (9.5%) and maternal diseases (26.2%). Some women did have more than one indication. Any side effects of aspirin were reported by 9.8% of the women. According to the SMAQ, 46.3% of the women was non-adherent to aspirin (Table 2).

According to the BBQ, 21.4% of the women was non-adherent to aspirin (Table 3). Five women (11.9%) were non-adherent according to both questionnaires, 14 women (33.3%) were non-adherent according to the SMAQ only and four women (9.5%) were non-adherent according to the BBQ only. One woman was adherent according to the BBQ, but did not answer all questions of the SMAQ (missing data).

No differences in demographic background or obstetrical characteristics between adherent and non-adherent women according to the SMAQ could be demonstrated (Table 4).

Table 1: Baseline characteristics of participants

	n=42	
		%
General characteristics		
Maternal age (years)	33.5 ± 3.9	
Non-Caucasian	10/37	27.0
Body mass index (kg/m2)	24.7 ± 12.5	
Alcohol use	2	4.8
Highest educational level		
Low	0	0.0
Middle	10	23.8
High	32	76.2
Obstetric history		
Parity	1.3 ± 0.8	
Progeniture	1.1 ± 0.7	
HD	22	52.4
FGR	16	38.1
IUFD	4	9.5
Indicated preterm birth <37 weeks gestation	28	66.7
Indicated preterm birth <34 weeks gestation	23	54.8
Gestational age at completing the survey		
Until 28 weeks	19/40	47.5
29-32 weeks	10/40	25.0
After 32 weeks	11/40	27.5

Data are depicted as mean ± SD or number and %. HD; hypertensive disorders of pregnancy, FGR; fetal growth restriction, IUFD; intrauterine fetal death.

Table 2: Responses to and non-adherence rates of the simplified medication adherence questionnaire (SMAQ).

Question	Response to the question (n=42)							
		%		%				
	Yes		**No**					
1. Do you ever forget to take your aspirin?	16	38.1	26	61.9				
2. Are you careless at times about taking your aspirin?	3	7.1	39	92.9				
3. Sometimes if you feel worse, do you stop taking your aspirin?	2	4.8	40	95.2				
4. Did you not take any of your aspirin over the past weekend?	1/41	2.4	40/41	97.6				
	Never		**1x**		**2-3x**		**4-5x**	**6-7x**
5. Thinking about the last week. How often have you not taken your aspirin?	33/41 80.5		5/41 12.2		1/41 2.4		1/41 2.4	1/41 2.4
	≤ 2 days		**> 2 days**					
6. Over the past 3 months, how many days have you not taken any aspirin at all?	35/40	87.5	5/40	12.5				
Non-adherence rate according to the SMAQ	19/41	46.3						

Data are depicted as number and %

Discussion

We found non-adherence rates for aspirin during pregnancy of 46.3% and 21.4% according to the SMAQ and BBQ respectively. No characteristics could be identified to recognize women at higher risk for non-adherence. To our knowledge, we are the first to report adherence rates of aspirin in pregnant women, which is of importance since aspirin is proven to be beneficial in the prevention of pregnancy complications like HD and FGR and its increased use in the obstetric field.

The non-adherence rates for aspirin in pregnant women in the present study are comparable with the non-adherence rates for aspirin in the non-pregnant population.[17;21] One could speculate that pregnant women could either be more adherent compared to non-pregnant patients because of clear short term goals in preventing recurrent pregnancy complications or less adherent because of fear to harm the fetus or possible side effect. The results of our study do not point into one of these two directions. Since we have examined women with high-risk pregnancies, one could speculate that these women are more adherent to aspirin compared to women with a milder indication for aspirin during pregnancy. Moreover, the examined population had frequent contact with their gynecologist, so one could suggest that in women with less frequent contact with the gynecologist, even more attention should be paid to adherence.

Looking more detailed into the answers women gave to the SMAQ and BBQ provides us information how we can increase adherence. More than one third of the women did ever forget to take a tablet of aspirin and almost 10% of the women forgot two or more tablets during one week. This knowledge should make the doctor aware that adherence cannot be taken for granted. Concerns about side effects occurred in about 10%, which should stimulate the caregiver to inform the women about the low prevalence and relatively mild side effects.

Other studies investigating adherence suggest that adherence can be enhanced by improvement of patient education and communication.[22;23] Improvement of patient communication includes options like sending reminders by e-mail or telephone and involving patients partners and/or family.[24] Moreover, a patient information leaflet can provide more information about the medication and correct intake. Pregnant women visit the outpatient clinic relatively often, so the caregiver can ask frequently if a woman takes her aspirin and can remind her to take it every day.

It is suggested that aspirin resistance, as a laboratorial diagnosis, is a contributing factor for recurrence of HD or FGR.[25;26] There neither is a definition, nor a golden standard to measure aspirin resistance. Poor adherence to aspirin has been suggested to be a cause of aspirin resistance.[27;28] To our knowledge, three studies and a review reported on aspirin resistance during pregnancy.[29-32] In a retrospective cohort study, the rate of HD was higher in women diagnosed with aspirin resistance, whose aspirin dosage was increased to 162 mg aspirin compared to women who did not need a dosage increase of aspirin.[29] Non-adherence could have played a role in the worse outcome in women in whom no effect of aspirin could be measured in their blood.

Strengths of this study include the use of validated questionnaires with satisfactory internal consistency, the high response rate and the prospective set-up in a high-risk population while still taking the medication.[18;20] Neither aspirin levels in serum can be measured reliably nor a golden standard to measure aspirin resistance exists, so validated questionnaires are the best way to examine adherence to aspirin, although self-report can give an underestimation of non-adherence.[33] To our knowledge, we are the first to report on non-adherence to aspirin in pregnant women. Limitations of the present study are the relatively small population and the fact that we did not check actual adherence by counting pills or timing of repeated prescriptions. The SMAQ and BBQ measure adherence in a different way, investigating either adherence or beliefs, experiences and behavior, resulting in different non-adherence rates. This study was not undertaken to investigate differences in outcomes between both questionnaires.

Conclusions

Almost half of pregnant women were found to be non-adherent to aspirin according to the SMAQ and almost a quarter according to the BBQ. The non-adherence rates in this population with high-risk for pregnancy complications shows that adherence cannot be taken for granted. The results of this study should increase doctor awareness that adherence can be improved and resulted in our hospital in a patient information leaflet.

Table 3: Results of Beliefs and Behaviour Questionnaire (BBQ, n = 42)

Statements	Strongly disagree		Disagree		Neutral		Agree		Strongly agree	
		%		%		%		%		%
Beliefs										
Confidence										
I have sufficient understanding about my illness	0	0.0	1	2.4	2	4.8	22	52.4	17	40.5
I know what to expect from my illness management	0	0.0	1	2.4	9	21.4	18	42.9	14	33.3
My current management will keep my illness at bay	0	0.0	2	4.8	11	26.2	22	52.4	7	16.7
I am receiving the best possible management	0	0.0	1	2.4	3	7.1	21	50.0	17	40.5
The management of my illness is a mystery for me	22	52.4	18	43.9	0	0.0	0	0.0	2	4.8
My aspirin is working	1	2.4	1	2.4	12	28.6	19	45.2	9	21.4
I have a say in the way my illness is managed	1	2.4	9	21.4	13	31.0	17	40.5	2	4.8
I have sufficient understanding about the options for managing my illness	0	0.0	0	0.0	2	4.8	19	45.2	21	50.0
My doctors are very knowledgeable	0	0.0	0	0.0	2	4.8	16	38.1	24	57.1
Concerns										
It is helpful to know the experiences of others with similar illness as mine	5	11.9	17	40.5	13	31.0	7	16.7	0	0.0
Natural remedies are safer than medicines	8	19.0	16	38.1	15	35.7	3	7.1	0	0.0
My doctors have limited management options to offer me	7/41	17.1	19/41	46.3	7/41	17.1	7/41	17.1	1/41	2.4
Using any medication during pregnancy involves some risk	3	7.1	15	35.7	13	31.0	11	26.2	0	0.0
I am on too many medications	19	45.2	16	38.1	4	9.5	3	7.1	0	0.0
Experiences										
Satisfaction										
My doctors are compassionate	0	0.0	0	0.0	9	21.4	24	57.1	9	21.4
I am satisfied with the information my doctors share with me	0	0.0	0	0.0	3	7.1	28	66.7	11	26.2
My doctors spend adequate time with me	0	0.0	0	0.0	1	2.4	27	64.3	14	33.3
Disappointment										
I am concerned about the side effects from aspirin during my pregnancy	13	31.0	23	54.8	2	4.8	4	9.5	0	0.0
It is unpleasant to use aspirin	15	35.7	22	52.4	3	7.1	1	2.4	1	2.4
It is physically difficult to handle aspirin	22	52.4	18	42.9	1	2.4	0	0.0	1	2.4
Financial difficulties limit my access to the best healthcare	29	69.0	12	28.6	0	0.0	0	0.0	1	2.4
The management of my illness disrupts my life	27	64.3	14	33.3	1	2.4	0	0.0	0	0.0

	Always		Often		Sometimes		Seldom		Never	
Behavior										
Adherence										
I have strict routines for using my aspirin	23	54.8	18	42.9	1	2.4	0	0.0	0	0.0
I keep my aspirin close to where I need to use them	31	73.8	9	21.4	0	0.0	2	4.8	0	0.0
I ensure I have enough aspirin so that I do not run out	27	64.3	9	21.4	6	14.3	0	0.0	0	0.0
I push myself to follow the instructions of my doctors	4	9.5	9	21.4	9	21.4	9	21.4	11	26.2
Non-adherence										
I get confused about my aspirin	0	0.0	0	0.0	3	7.1	13	31.0)	26	61.9)
I make changes in the recommended management to suit my lifestyle	0	0.0	2	4.8	1	2.4	6	14.3)	33	78.6)
I vary my recommended management based on how I am feeling	0	0.0	0	0.0	0	0.0	2	4.8)	40	95.2)
I put up with my medical problems before taking any action	4	9.5	11	26.2	14	33.3	9	21.4)	4	9.5)
Non-adherence rate according to BBQ	9	21.4								

Data are depicted as number and %

Table 4: Maternal characteristics between adherent and non-adherent women according to the simplified medication adherence questionnaire (SMAQ).

	Adherent n=27)		**Non-adherent n=14)**		***p*-value**
	%		%		
Age (years)	32.8 ± 3.8		34.3 ± 4.0		0.23
Non-Caucasian	5	50.0	5	50.0	1.00
Body mass index (kg/m2)	24.8 ± 4.3		24.8 ± 5.4		0.99
Educational level					0.47
Middle	6	66.7	3	33.3	
High	16	50.0	16	50.0	
Gestational age completing survey	29.2 ± 3.8		30.7 ± 5.3		0.32
Indication for aspirin					
HD	13	59.1	9	40.9	0.54
FGR	9	56.3	7	43.8	0.79
IUFD	2	40.0	3	60.0	1.00
Maternal diseases	4	40.0	6	60.0	0.47

Data are depicted as mean ± SD or number and %
HD = hypertensive disorders of pregnancy, FGR = fetal growth restriction, IUFD = intrauterine fetal death

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Post-pregnancy aspirin resistance appears not to be related with recurrent hypertensive disorders of pregnancy

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Abstract

Objective: The FRUIT-RCT concluded that low-molecular-weight heparin added to aspirin compared to treatment with aspirin alone is beneficial in the prevention of early-onset hypertensive disorders of pregnancy (HD) in women with inheritable thrombophilia and prior HD and/or a small-for-gestational age (SGA) infant leading to delivery before 34 weeks gestation. The aim of this study is to answer the question whether aspirin resistance is associated with recurrent HD.

Study Design: Women with and without recurrent HD matched for age, study arm, and chronic hypertension were invited for this follow-up study 6-16 years after they participated in the FRUIT-RCT. Aspirin resistance was tested after 10 days of aspirin intake using three complementary tests: PFA-200, VerifyNow® and serum thromboxane B_2 (TXB_2). An independent t-test, Mann-Whitney U test, Fisher's Exact test and Chi² test were used for the statistical analyses.

Results: Thirteen of 24 women with recurrent HD and 16 of 24 women without recurrent HD participated. The prevalence of laboratory aspirin resistance was 34.5% according to the PFA-200, 3.4% according to the VerifyNow® and 24.1% according to TXB_2. The prevalence of aspirin resistance by any test was 51.7%. Aspirin resistance per individual test did not differ between women with and without recurrent HD. Aspirin resistance measured by any test occurred more frequently in women without recurrent HD ($p<0.01$), irrespective of low-molecular-weight heparin.

Conclusions: No relation could be demonstrated between recurrent HD and aspirin resistance per test, measured up to 16 years after pregnancy. On the contrary, complementary aspirin resistance measurements were encountered more frequently in women without recurrent HD.

Introduction

After a recent trial, concluding that low-molecular-weight heparin when added to aspirin could potentially prevent early-onset hypertensive disorders of pregnancy (HD: preeclampsia, eclampsia and HELLP-syndrome) in women with inheritable thrombophilia and adverse obstetric history, a new research question arose.[1] In a letter to the editor Bujold et al questioned whether the effect of low-molecular-weight heparin could have been mainly beneficial for the subgroup of women resistant to aspirin.[2] The term aspirin resistance is under debate, nowadays a term like aspirin non-responsiveness is also used.
About one third of cardiovascular high risk patients and trauma patients have been shown to be resistant to aspirin.[3-8] This is in line with the prevalence of aspirin resistance been found in women taking aspirin during pregnancy.[9-11] Two studies concluded that adjusting aspirin dosage might be needed to prevent HD in high risk women.[9;10] A third study concluded that aspirin resistance might be related to the occurrence of HD. [11] However, the relation between aspirin resistance and the occurrence or recurrence of HD has not been well explored. Elucidating this relation could lead to more individualized treatment in women who use aspirin during pregnancy and thereby improve their pregnancy outcomes. The aim of this study is to explore whether there is a relation between aspirin resistance and recurrent HD in the currently non-pregnant women who participated in the FRactionated heparin in women with Utero-placental Insufficiency and Thrombophilia randomized controlled trial (FRUIT-RCT)?[1]

Materials and Methods

Participants
This study is a follow-up study of the FRUIT-RCT[1] In the FRUIT-RCT, 139 women were included in the Netherlands (n=126), Sweden (n=3) and Australia (n=10) between January 2000 and December 2009. All women had previous HD and/or a SGA-infant, delivered before 34 weeks gestation and had inheritable thrombophilia. Women were randomized to the combination of low-molecular-weight heparin and aspirin, or aspirin alone. For the current follow-up study, performed 6-16 years after women joined the FRUIT-RCT, we invited all participants from the Dutch subsample with HD during their study pregnancy (n=24) and 24 matched for age, study arm and chronic hypertension without HD. Since the effect of aspirin resistance on the presence of HD is unknown, a power calculation could not be performed. Therefore, we invited all women with recurrent HD during the FRUIT-RCT to provide information for future studies. Exclusion criteria were: diabetes mellitus, use of drugs known to alter platelet function (e.g. NSAID's, beta-lactam antibiotics, SSRI's and amitriptyline, chronic use of antiplatelet agents) within two weeks before enrollment, major surgical procedure within one week before enrollment, a cardiovascular event within three months before enrollment and abnormal cell counts of hemoglobin, hematocrit, leucocytes and/or thrombocytes. The study was conducted in accordance with the Helsinki II Declaration and was approved by the Institutional Review Board of the VU University Medical Center in Amsterdam, the

Netherlands. The study was registered at the Dutch Trial Register (Nederlands Trialregister; www.trialregister.nl) with number NTR5106. After written informed consent, we visited all women in their own local hospital.

Measurements

An update of the medical history after the FRUIT-RCT pregnancy, current use of medication and lifestyle habits was obtained via a questionnaire. Participants were instructed to take one tablet of aspirin (acetylsalicylic acid, 80mg, non-enteric-coated) at 8pm for ten days. Venous blood samples were collected after an overnight fast at 8am. Blood samples were taken on two separate days; on day one, before usage of aspirin, and on day eleven, after ten days of aspirin intake. On day one, aspirin resistance was measured alongside with hemoglobin, hematocrit, thrombocytes and leucocyte counts. On day eleven two blood samples were collected within a timeframe of five minutes, to analyze aspirin resistance. The mean of these two results on day eleven was used in the analysis. Aspirin resistance was tested with three different tests: Platelet Function Analyzer-200 (PFA-200, INNOVANCE® PFA-200 System, Siemens Healthcare, Marburg, Germany); the VerifyNow® point-of-care system (Accumetrics, CA, USA); and serum thromboxane B_2 (TXB_2) level using an enzyme immunoassay kit (Assay Designs®, Ann Arbor, MI, USA).

PFA-200 measures the process of primary hemostasis.[12] For the analysis, citrated whole blood is passed through a capillary. The system measures platelet plug formation; the capillary will occlude. The time needed for complete obstruction of the capillary is the closure time (CT). A CT of ≤150 seconds was used for the dichotomous definition of aspirin resistance.[9,10] PFA has a theoretical maximum of 300 seconds which means that any CT >300 seconds is reported as 301 seconds. The Collagen/Epinephrine cartridge was used.

VerifyNow® utilizes arachidonic acid as an agonist to measure the antiplatelet effect of aspirin specifically along the pathway of inhibition of COX-1. A small tube of whole blood is inserted into an aspirin cartridge. The cartridges including the tube are inserted into VerifyNow® to measure the change in light transmittance through a patient's blood sample which results into Aspirin Reaction Units (ARU). [13] An ARU of ≥550 was used for the dichotomous definition of aspirin resistance.[14,15] The aspirin cartridge was used.

Serum TXB_2 is a direct measure of the capacity of platelets to synthesize TXA_2 and a specific measure of the pharmacological effect of aspirin on platelets [16]. Directly after blood collection, blood samples were placed in a stove for one hour at 37 degrees Celsius. After one hour, the blood samples were centrifuged for 10 minutes with 3000 rotations per minute. All serum samples were stored at -80 degrees Celsius and analyzed within six months in the laboratory for hematology, unit thrombosis and hemostasis of the Radboud University Medical Center in Nijmegen, the Netherlands. A TXB_2 concentration above the highest quartile was defined as aspirin resistant.[11]

Statistics

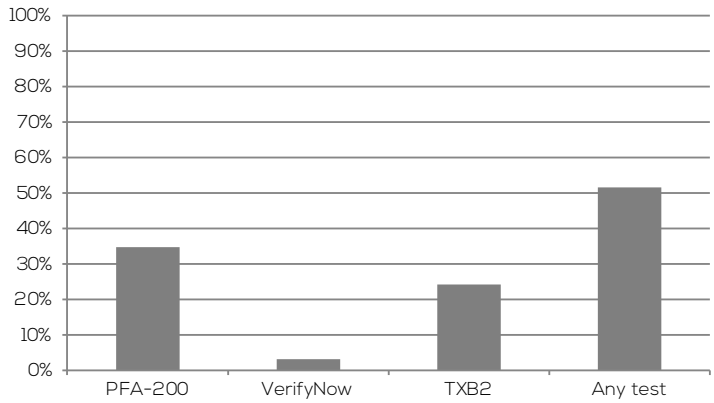
Baseline characteristics between the group with and without HD during the FRUIT-RCT were examined using an independent t-test, Mann-Whitney U test, or Chi^2 test. Results of

different devices were given in both continuous and dichotomized outcomes. Differences in aspirin resistance between women with and without HD were tested with an independent t-test, Mann-Whitney U test, Fisher's Exact test, or Chi^2 test was used. Moreover, aspirin resistance occurrence was also examined taking into account the treatment arm during the FRUIT-RCT and examined between four groups: women with and without HD and with or without low-molecular-weight heparin treatment during the FRUIT-RCT using a Fisher's Exact test or Chi^2 test.
Statistical analyses were performed with IBM SPSS version 22.0 (SPSS Inc, Chicago, USA). A two-tailed *p*-value <0.05 was considered to be significant.

Results

From the 48 women, two died (one from a cerebral lymphoma, one from a cerebral aneurysm), five were lost to follow-up and 12 refused participation. Twenty-nine participated, 13 with and 16 without recurrent HD. Four of the six women who had early-onset recurrent HD during the FRUIT participated. There was no difference in obstetrical history between de 29 participants and women who did not participate (data not shown).
There were no differences in baseline characteristics between the groups (Table 1).
We asked during the second visit whether all ten tablets of aspirin were taken and every participants confirmed this. The results of aspirin resistance in relation to recurrence of HD during FRUIT-RCT are presented in Table 2. The prevalence of aspirin resistance per device is depicted in Figure 1.
Differences in aspirin resistance per device was not statistically significant between women with and without HD taking into account low-molecular-weight heparin usage during the FRUIT-RCT (p=0.20 for PFA-200, p=0.41 for VerifyNow®, p=0.88 for TXB_2). Aspirin resistance measured by any test did differ (p=0.04) between the four groups (Figure 2).

Figure 1: Prevalence of aspirin resistance per device (n=29)



PFA-200; Platelet Function Analyzer-200, TXB_2; thromboxane B_2

Table 1: Baseline characteristics

	HD during FRUIT-RCT (n=13)	No HD during FRUIT-RCT (n=16)	*p-value*
Age at follow-up	44.7 ± 3.3	42.9 ± 4.7	0.26
Non-Caucasian	1 (7.7)	2 (12.5)	1.00
BMI at follow-up	27.8 ± 6.0	27.1 ±5.5	0.74
Smoking at follow-up	2 (15.4)	3 (18.8)	1.00
Thrombophilia disorder*			
Protein S deficiency	1 (7.7)	2 (12.5)	1.00
Protein C deficiency	3 (23.1)	1 (6.3)	0.30
Factor V Leiden	9 (69.2)	8 (50.0)	0.30
APCr	0 (0)	1 (6.3)	1.00
Factor II mutation	2 (15.4)	6 (37.5)	0.24
Years since FRUIT-RCT pregnancy	9.0 ± 2.5	9.1 ± 3.1	0.97
Pregnancy outcomes FRUIT-RCT			
Treatment with low-molecular-weight heparin and aspirin (others were treated with aspirin only)	8 (61.5)	9 (56.3)	0.77
SGA infant outcome pregnancy	3 (23.1)	3 (18.8)	1.00
GA delivery outcome pregnancy (days)	246.8 ± 41.8	267.4 ± 22.4	0.13
Hemoglobin mmol/L	8.9 ± 0.8	8.5 ± 0.8	0.20
Hematocrit L/L	0.42 ± 0.03	0.40 ± 0.03	0.17
Thrombocytes (10^9/L)	265.4 ± 61.0	261.0 ± 58.8	0.85
Leucocytes (10^9/L)	6.8 ± 1.3	6.6 ± 1.7	0.72

HD; hypertensive disorders of pregnancy, FRUIT-RCT: FRactionated heparin in women with Utero-placental Insufficiency and Thrombophilia randomized controlled trial1, BMI; body mass index, APCr; Activated protein C resistance, SGA; small-for-gestational age, GA; gestational age. *Some women had more than one diagnosis.
Data are depicted as mean ± SD or number (%).

Comment

Evaluating the results of this cohort of thrombophilic women, we did not demonstrate a relation between aspirin resistance and recurrent HD measured by any device. On the contrary, the complementary aspirin resistance measurements were encountered more frequently in women without recurrent HD. We do not have an explanation why aspirin resistance occurred more often in women without recurrent HD is not in line with our hypothesis. To our knowledge, only three other studies and one review reported on aspirin resistance in relation to pregnancy outcomes.[9-11,17] In the largest study (n=270), a retrospective cohort study, lower rates of HD were found in women who were monitored during pregnancy by the PFA-100. The rate of HD was higher in women diagnosed with aspirin resistance, whose aspirin dosage was increased to 162 mg aspirin compared to women who did not need a dosage increase of aspirin.[9] The authors concluded that platelet function testing and individualized dosing of aspirin might be effective in preventing HD in high risk women. A limitation of this study is that the dosage of aspirin was increased immediately when women were found to be resistant to aspirin. In our opinion, a first step would be to examine whether aspirin resistance is related to occurrence of HD. In a small cohort study (n=43) in which TXB_2 levels in urine were measured, a relation between aspirin resistance and HD was suggested.[11] In contrast, another prospective cohort study (n=87), demonstrated no differences in obstetric complications (not further specified) between women who were and were not resistant to

Table 2: Main results: aspirin resistance in relation to recurrence of HD during FRUIT-RCT

	HD during FRUIT-RCT (n=13)	**No HD during FRUIT-RCT** (n=16)	***p-value***
PFA-200 CT day 1	135.7 ± 56.8	121.4 ± 60.6	0.52
PFA-200 mean CT day 11	242.5 [162.5-284.3]	151.5 [127.0-257.3]	0.10
PFA-200 difference between day 1 and 11	89.15 ± 67.7	64.72 ± 79.4	0.38
PFA-200 aspirin resistant	2 (15.4)	8 (50.0)	0.11
VerifyNow ARU day 1	639.0 [615.5-650.0]	637.5 [623.8-654.3]	0.81
VerifyNow mean ARU day 11	402.0 [392.5-461.0]	418.8 [407.4-434.3]	0.33
VerifyNow difference between day 1 and 11	193.5 [122.5-253.0]	212.5 [201.1-237.4]	0.81
VerifyNow aspirin resistant	0 (0)	1 (7.3)	1.00
TXB_2 ELISA day 1	974.9 ± 608.5	1281.0 ± 673.0	0.22
Mean TXB_2 ELISA day 11	9.4 ± 4.9	12.8 ±9.2	0.24
TXB_2 ELISA difference between day 1 and 11	965.6 ± 607.0	1268.2 ± 668.0	0.22
TXB_2 aspirin resistant	2 (15.4)	5 (31.3)	0.41
Aspirin resistant by any test	3 (23.1)	12 (75.0)	0.005

HD; Hypertensive disorder of pregnancy, FRUIT-RCT: FRactionated heparin in women with Utero-placental Insufficiency and Thrombophilia randomized controlled trial1, PFA-200; Platelet Function Analyzer-200, CT; closure time, ARU; aspirin reaction units, TXB2; thromboxane B2.
A CT of ≤150 seconds was used for the dichotomous definition of aspirin resistance 9;10
An ARU of ≥550 was used for the dichotomous definition of aspirin resistance 14;15.
A TXB2 concentration above the highest quartile was defined as aspirin resistant 11.
Data are depicted as mean ± SD, number (%), or median [IQR].

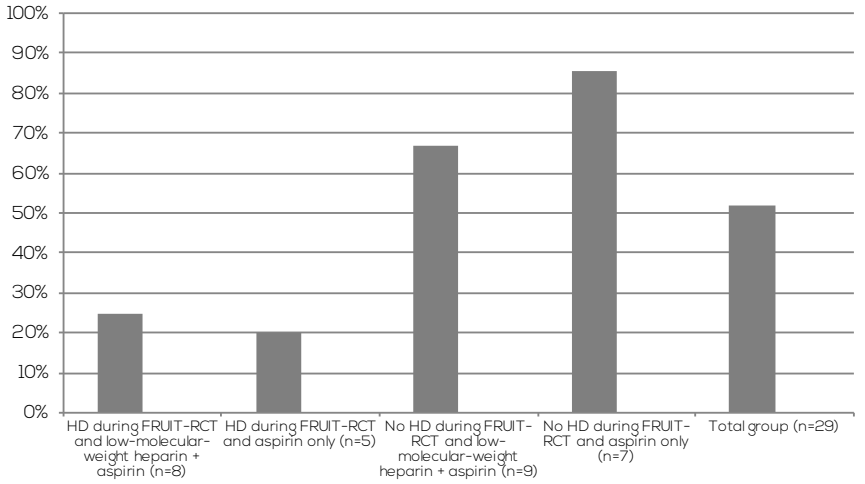
aspirin according to the PFA-100.[10]

Returning to Bujold et al's[2] question, we do not even see a trend into the suggestion that low-molecular-weight heparin was beneficial in the subgroup of women who are resistant to aspirin We realize that our study is under-powered to provide a firm conclusion.

Another limitation is that it is unknown whether aspirin resistance changes over time and how pregnancy affects aspirin resistance. Platelet activity might be increased during pregnancy compared to non-pregnant women (although these results are not unanimous) and is even more altered in women with HD compared to women without HD due to impaired uteroplacental circulation.[18-20] This might suggest that the more platelet activity the more aspirin resistance. Extending this leads to the premise that women diagnosed as aspirin resistant outside pregnancy are also aspirin resistant during pregnancy. Women not resistant to aspirin outside pregnancy, could still be resistant to aspirin during pregnancy. To investigate this, our next step is to evaluate whether aspirin resistance changes over time during pregnancy and thereafter. Poor adherence to aspirin has been suggested to be a cause of aspirin resistance, but the participants of this study confirmed the intake of the ten tablets.[4;21] Finally, one should take into account that aspirin resistance is a laboratorial result and not a clinical diagnosis.

A strength of the present study is the use of three complementary devices, investigating different aspects of platelet function. Reassuring is that the aspirin resistance measurements were convincingly different without aspirin usage on day one and with aspirin usage on day

Figure 2: Prevalence of aspirin resistance by any test, taking presence of HD and low-molecular-weight heparin usage during the FRUIT-RCT into account



HD; Hypertensive disorder of pregnancy, FRUIT-RCT: FRactionated heparin in women with Utero-placental Insufficiency and Thrombophilia randomized controlled trial[1]

eleven. All three devices taken together suggest that aspirin resistance is less encountered in women with recurrent HD. This study does not show which device is most reliable; further research is needed to identify the most reliable way to determine aspirin resistance. The prevalence of aspirin resistance in this study varied from 3.7% to 32.7% depending on which device was used, with the lowest prevalence measured by VerifyNow® and the highest prevalence measured by PFA-200 which is in line with another study.[22] Our results suggest that, due to the differences in outcomes between the different devices, studies should use more than one device to examine aspirin resistance.

Another strength is that we followed a strict protocol to minimize external influences on the measurements, since platelet activity is influenced by, among other things, medication, time of blood draw and diet.[23] Furthermore, due to the prospective set-up of this study the obstetric history is thoroughly documented.

In conclusion, no relation between recurrent HD and aspirin resistance measured by any device could be demonstrated. On the contrary, complementary aspirin resistance measurements were encountered more frequently in women without recurrent HD.

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8

Resistance of Aspirin During and After pRegnancy; protocol of the RADAR study, a longitudinal cohort study

Study in progress

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Abstract

Background: Previously, a study was performed which demonstrated no relation between aspirin resistance and hypertensive disorders of pregnancy (HD). However, aspirin resistance was measured 6-16 years after pregnancy. The present study is undertaken to assess the consistency of aspirin resistance during and after pregnancy.

Patients and methods: We perform a longitudinal cohort study including women who have an indication for aspirin during pregnancy. Aspirin resistance is measured in the first, second and third trimester of pregnancy and at least three months postpartum. Four complementary tests are used: PFA-200, VerifyNow®, Chrono-log light transmission aggregometry and serum thromboxane B_2.

Results: Out of 38 invited women, 14 declined participation and 1 had a spontaneous miscarriage, resulting in 23 participants in this study (participation rate 60.5%). We expect that the last postpartum measurements will be performed in January 2017. The results are expected during the first half of 2017. Adherence to aspirin usage was investigated by asking at the moment of blood sampling.

Discussion: It is unknown whether aspirin resistance changes over time and how pregnancy affects aspirin resistance. This study will elucidate how aspirin resistance develops over time during the three trimesters of pregnancy and after pregnancy. The results will support us with the interpretation of the results of our previously performed study on the possible relation between aspirin resistance and HD. Adding the knowledge of the present study with the use of several complementary tests facilitates comparison with other studies and thus might give more insight if aspirin resistance is a factor of importance in the treatment to prevent HD.

Background

A multicenter randomized controlled trial (RCT) the FRUIT-RCT, concluded that addition of low-molecular-weight heparin (LMWH) to the standard care aspirin is beneficial in the prevention of early-onset (<34 weeks gestation) hypertensive disorders of pregnancy (HD) in women with inheritable thrombophilia and adverse obstetric history.[1] It was suggested that the effect of LMWH could have been mainly beneficial in the subgroup of women who are resistant to aspirin.[2] Aspirin resistance is currently becoming a slightly outdated term. Nowadays a term like laboratorial aspirin non-responders is used more often. Still, we chose to use the term aspirin resistance, because it is the most well know term at least in the small field of obstetrics.

Aspirin binds to cyclooxygenase-1 inhibiting steps in the conversion of arachidonic acid to thromboxane A2 (TXA_2), by which it inhibits platelet activation. Aspirin resistance is the phenomenon of a less effective response to aspirin in some patients. The prevalence of aspirin resistance in populations of man and non-pregnant women with cardiovascular diseases is about 20%.[3,4] A literature search revealed three studies and one review reporting on aspirin resistance during pregnancy, showing that around one third is resistant to aspirin.[5-8] Timing of measurement, a possible confounder, in the three studies was not standardized.[5-7]

A follow-up study of the FRUIT-RCT was performed to investigate if there is a relation between HD and aspirin resistance (submitted, Chapter 7). Conclusion of this study was that no relation could be demonstrated between recurrent HD and aspirin resistance. This was measured using three complementary tests after 10 days of aspirin use. The limitation of this study is that aspirin resistance was measured up to 16 years after pregnancy.

In the present study, the RADAR study (Resistance of Aspirin During and After pRegnancy), aspirin resistance will be examined during the three trimesters of pregnancy and more than three months postpartum in a population of women who have an indication for aspirin. Aspirin resistance will be examined with four complementary tests. The results of the RADAR study are necessary to conclude if the occurrence of aspirin resistance in the non-pregnant women of the FRUIT-RCT is representative for aspirin resistance during pregnancy. Various factors may account for changes in the aspirin resistance over time during the three trimesters of pregnancy: the plasma volume expansion throughout the first and second trimester, change in leukocyte and thrombocyte count during pregnancy and change in steroid hormones during pregnancy.[9-11] All these factors can have an effect on pharmacokinetics and/or pharmacodynamics and thus explain differences in occurrence of aspirin resistance in the different periods during pregnancy.

The following questions will be answered in this longitudinal cohort study:

1. Is the prevalence of aspirin resistance consistent between the first, second and third trimester of pregnancy?

2. Is aspirin resistance after pregnancy related to aspirin resistance during pregnancy?

To our knowledge, neither aspirin resistance during the three trimesters of pregnancy nor

consistency of aspirin resistance during and after pregnancy has been examined using four complementary tests.

Patients and methods

Knowledge synthesis planning

The group of principal investigators; practicing clinicians, obstetricians and internal medicine specialists and a pharmacist active in the aspirin research area, met during several meetings for the set-up of the study. During these meetings eligibility criteria and instructions for participants to reduce the chance of bias in the measurements were discussed. Moreover, the procedure of measurements were determined. These items are described in detail below. The present study was carefully carried out by a small team of well instructed people (CNHA, JMbdW and two well-trained students: Arda Arduç and Omar Hammad). Since no golden standard to examine aspirin resistance in pregnant women exists, we could not perform a power calculation. We planned to evaluate changes in aspirin resistance over time in twenty women during pregnancy and three months postpartum which is in analogy to other aspirin resistance studies.[12]

Eligibility criteria

In- and exclusion criteria are depicted in Table 1. All patients were recruited in the VU University Medical Center, Amsterdam, the Netherlands. Obstetric and general medical history had been obtained through medical chart reviewing. Doctors in the outpatient clinic informed patients about the RADAR study and provided the patient information letter, thereafter patients were phoned by one of the investigators to explain the purpose of this study and answer questions. After signed informed consent the women were included. The study was conducted in accordance with the Helsinki II Declaration and was approved by the Institutional Review Board of the VU University Medical Center in Amsterdam, the Netherlands. The study was registered at the Dutch Trial Register (Nederlands Trial register; www.trialregister.nl), NTR5106.

Instructions for participants

A flow-chart of the study is presented in Figure 1. Measurements were scheduled during the first trimester of pregnancy between 10 and 15 weeks gestation, in the second trimester between 18 and 26 weeks gestation and in the third trimester between 28 and 36 weeks gestation. The postpartum measurement was scheduled at least three months after delivery. Participants were instructed to take aspirin (acetylsalicylic acid, 80mg, non-enteric-coated) in the evening and ten days before their appointment strict at 8pm. Venous blood samples were collected after an overnight fast at 8am and participants were instructed not to smoke 30 minutes prior to the appointment. Besides aspirin resistance measurements, blood sampling was performed for hemoglobin, hematocrit, thrombocytes and leucocyte counts. During the visit in the first trimester, a physical examination including blood pressure, body

Table 1: In- and exclusion criteria of the RADAR study

Inclusion criteria	Exclusion criteria
• 18 years or older	• Systemic Lupus Erythematosus
• Understand Dutch or English	• Antiphospholipid syndrome
• Obstetrical and other indications for aspirin during pregnancy	• Poorly controlled hypertension before pregnancy or in first trimester
	• Hypercholesterolemia before pregnancy or in first trimester
	• Impaired renal or liver function before pregnancy or in first trimester
	• Recent cardiovascular event <3 months
	• Abnormal cell count
	• Drugs that are known to alter platelet function; e.g. NSAID's, tirofiban, eptifibatide, abciximab, beta-lactam antibiotics, dextran, SSRI's, clomipramine & amitriptyline, dipyridamole, verapamil, diltiazem , ginkgo biloba, ginseng, St John's wort.

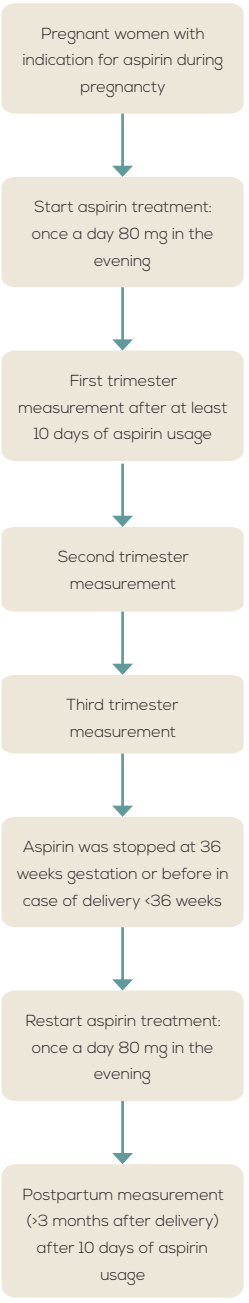
weight and length measurements were performed and a survey about family history was answered. Adherence to aspirin usage was investigated by asking at the moment of blood sampling and written down in the case record form. During the postpartum visit blood pressure and body weight were measured again.

Procedure of measurements

Aspirin resistance is examined during the first, second, third trimester of pregnancy and postpartum (after taking aspirin for another 10 days). Until now, there is no gold standard test for aspirin resistance.[13] Therefore, four complementary tests are used to investigate different aspects of platelet function: 1.Platelet Function Analyzer-200 (PFA-200, INNOVANCE® PFA-200 System, Siemens Healthcare, Marburg, Germany); 2. VerifyNow® point-of-care system (Accumetrics, CA, USA); 3.Chronolog light transmission aggregometry (LTA) and 4. Serum thromboxane B_2 (TXB_2) level using an enzyme immunoassay kit (Assay Designs®, Ann Arbor, MI, USA).

1. PFA-200 measures the process of primary hemostasis.[14] For the analysis, citrated whole blood is passed through a capillary. The system measures platelet plug formation; the capillary will occlude. The time needed for complete obstruction of the capillary is the closure time (CT). A CT of ≤150 seconds is used for the dichotomous definition of aspirin resistance.[5,6] PFA has a theoretical maximum of 300 seconds which means that any CT >300 seconds is reported as 301 seconds. The Collagen/Epinephrine cartridge is used.

2. VerifyNow® utilizes arachidonic acid as an agonist to measure the antiplatelet effect



of aspirin specifically along the pathway of inhibition of COX-1. A small tube of whole blood is inserted into an aspirin cartridge. The cartridge including the tube are inserted into VerifyNow® to measure the change in light transmittance through a patient's blood sample which results into Aspirin Reaction Units (ARU).[15] An ARU of ≥550 is used for the dichotomous definition of aspirin resistance.[16,17]The aspirin cartridge is used.

3. Chronolog LTA measures the light transmittance of platelet rich plasma which is influenced by platelet aggregation stimulated by arachidonic acid.[18] Arachidonic acid reduces the production of TXA_2 which results in less platelet aggregation. If women were resistant to aspirin, more aggregation occurs and the light transmittance is increased. The percentage of maximal aggregation is measured and a value >22% is defined as aspirin resistant.

4. Serum TXB_2 is a direct measure of the capacity of platelets to synthesize thromboxane A_2 and a specific measure of the pharmacological effect of aspirin on platelets.[19] Directly after blood collection, blood samples are placed in a stove for one hour at 37 degrees Celsius. After one hour, the blood samples are centrifuged for 10 minutes with 3000 rotations per minute. All serum samples are stored at -80 degrees Celsius and will be analyzed in the laboratory for hematology, unit thrombosis and hemostasis of the Radboud University Medical Center in Nijmegen, the Netherlands. A TXB_2 concentration above the highest quartile is defined as aspirin resistant.[7]

Figure 1: Flow-chart of the RADAR study

Outcomes

Baseline characteristics include age, number of previous pregnancies, body mass index before pregnancy, blood pressure in the first trimester, smoking behaviour, alcohol use, indication for aspirin usage, obstetric history and family history including vascular diseases and HD. Main outcome of this study is aspirin resistance measured using the PFA-200, VerifyNow,

Chronolog LTA and serum TXB_2. Both the continuous data as well as the dichotomized data regarding aspirin resistance are examined. The results of each test separately is examined, and additionally occurrence of aspirin resistance in one of the four tests, labelled as 'resistant by any device'.

Statistics

Outcomes of the four complementary tests will be compared with one-way ANOVA or paired samples t-test when normally distributed and Friedman test or Wilcoxon signed rank test when not normally distributed. Dichotomous data will be analysed with a Cochran's Q-test. SPSS version 22.0 (SPSS Inc., Chicago, IL, USA) is used to perform the statistical analyses. A two-tailed *p*-value <0.05 is considered to be significant.

Results

Eligible were 38 women. Fourteen women declined participation. One women dropped out of the study because of a spontaneous miscarriage, resulting in 23 participants in this study (participation rate 60.5%). Mean maternal age at start of pregnancy was 35.1 ± 3.4. Indications for aspirin usage in pregnancy were previous HD, intrauterine fetal death, small-for-gestational age infant and/or chronic hypertension.

We expect that the last postpartum measurements will be performed in January 2017. The results are expected in the first half of 2017.

Discussion

This longitudinal cohort study will investigate consistency of aspirin resistance during pregnancy and examines if aspirin resistance after pregnancy is related to aspirin resistance during pregnancy.

It is unknown whether aspirin resistance changes over time and how pregnancy affects aspirin resistance. Various factors may play a role. First, platelet activity might be increased during pregnancy compared to non-pregnant women. This applies to uncomplicated pregnancy, but is even more altered in women with HD due to impaired utero-placental circulation and therefore endothelial damage.[20-22] One could suggest that the more platelet activity the more aspirin resistance. Extending this thought leads to the premise that women diagnosed as aspirin resistant outside pregnancy are also aspirin resistant during pregnancy. Women not resistant to aspirin outside pregnancy, could still be resistant to aspirin during pregnancy. Second, other influences like the physiological plasma volume expansion and change in steroid hormones during pregnancy might play a role as mentioned in the introduction.

The present study will give more insight in the results of the follow-up study of the FRUIT-RCT which found no relation between aspirin resistance and HD (submitted, Chapter 7). In this follow-up study, aspirin resistance was measured 6-16 years postpartum and the results

of the present study will inform us if these measurements are representative for the aspirin resistance status during pregnancy. The knowledge of the present study will also elucidate whether reliable aspirin resistance testing might be feasible and as such of importance in the treatment to prevent HD. Tailored medicine according to aspirin resistance status, might become an aspect of importance in the treatment with aspirin during pregnancy.

The instruction to take aspirin during the evening is given to achieve a stable level of aspirin in the serum and thereby a more stable 24-hours platelet inhibition.[12] Time dependant effect of aspirin intake has been acknowledged more than a decade within obstetrical application and less complications including HD and fetal growth restriction (FGR) are described when women take their aspirin in the evening.[23-26]

Adherence to aspirin is measured since poor adherence to aspirin has been suggested to be a cause of aspirin resistance.[13;27] Our group has examined the adherence to aspirin in a population with an indication for aspirin during pregnancy. Despite the mainly obstetrical indications in a well-informed population we found adherence rates of 53.7-78.6%.[28]

Furthermore, the timing of the appointment, overnight fast and prohibition to smoke [30] minutes before the appointment are chosen since these factors influence platelet activity.[29]

The strength of this study is its novelty; to our knowledge, neither aspirin resistance in pregnant women using four complementary tests has been investigated before, nor consistency of aspirin resistance during and after pregnancy with multiple tests. Studies using multiple tests are needed to explore associations with clinical outcome parameters, to examine which device is reliable for clinical purpose, since no golden standard to measure aspirin resistance exists.[13] Another strength of this study is its prospective and longitudinal set-up.

The limitation of this study is that it is focused on the evaluation of the various tests. We will evaluate pregnancy outcomes, but unfortunately this study is not powered to measure the influence of aspirin resistance hereupon.

Depending on the results of the present study, we might incorporate aspirin resistance measurements in a recently started RCT. This RCT investigates if aspirin is beneficial in the prevention of preterm birth in women who are randomized into aspirin or placebo (L. Visser et all. Low dose aspirin in the prevention of recurrent spontaneous preterm labour – the APRIL study: a multicenter randomized placebo controlled trial. Submitted). This RCT will give the opportunity to compare platelet activity between women with and without aspirin usage. No relation between pregnancy complications (not further specified) and aspirin resistance according to the PFA-100 was described, although statistical comparison was lacking due to small size of the groups.[5] Future research should evaluate if a correlation with adverse pregnancy outcome and aspirin resistance exists, a suggestion which is also made in a recent review.[8]

In conclusion, by means of this study we hope to support the knowledge on consistency of aspirin resistance during and after pregnancy.

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Cardiovascular risk factors in women with inheritable thrombophilia a decade after single or recurrent hypertensive disorder of pregnancy

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Abstract

Objective: To described cardiovascular risk factors in women with inheritable thrombophilia 8-19 years after early-onset hypertensive disorders of pregnancy (HD) with or without recurrent HD.

Methods: Women with recurrent HD were compared with women with single HD, for physical examination and cardiovascular parameters in serum.

Results: Systolic blood pressure, diastolic blood pressure and albumin creatinine ratio were higher in women with recurrent HD compared to women with single HD (p=0.046, p=0.029 and p=0.008 respectively). In both groups 72.7% had an increased cardiovascular risk.

Conclusion: Women with inheritable thrombophilia after single or recurrent HD have a high cardiovascular risk.

Introduction

In the Western world, cardiovascular disease (CVD) is the number one cause of death in women[1] It is a commonly accepted theory that pregnancy is a stress test which can identify women who have an elevated risk to develop CVD.[2-5] It has not been elucidated yet whether hypertensive disorders of pregnancy (HD) is a disease within the CVD spectrum or that HD itself is a risk factor for CVD later in life, comparable with other risk factors such as hypertension, hypercholesterolemia and obesity.[6;7]

The risk to develop cardiovascular risk factors has been reported to be related to the HD severity.[2;8] In retrospective studies, it also is suggested that women with a history of recurrent HD have a higher chance to develop CVD later in life compared to women with a history of single HD.[9;10]

t is unknown whether inheritable thrombophilia is an additional risk factor next to HD for CVD.[11-13] Whether inheritable thrombophilia is related to HD and therefore indirectly linked to CVD, has been debated. Studies, including meta-analyses, taken the severity and time of onset of HD into account, show an association between inheritable thrombophilia and pregnancy complications like early-onset HD (HD < 34 weeks gestation) and small-for-gestational age (SGA) infants.[14-18] Two case-control studies and one cohort study including a meta-analysis, conclude that there is no relation between inheritable thrombophilia and pregnancy complications.[19-21] Only one of the case-control studies took severity and time of onset of HD into account.[20]

One study investigated cardiovascular risk factors in women with thrombophilia more than five years after a pregnancy complicated by HD and concluded that thrombophilia might mediate in lowering cardiovascular risk factors compared to women without thrombophilia with a similar obstetric history.[22]

We hypothesize that women with a history of recurrent HD develop more cardiovascular risk factors compared to women with a history of single HD.

We have the unique opportunity to investigate cardiovascular risk factors in women with inheritable thrombophilia 8-19 years after early-onset HD with or without recurrent HD, whose pregnancy outcomes were well documented. The specific question is: is a history of recurrent HD associated with increased incidence of cardiovascular risk factors compared to a history of single HD?

Patients and methods

Participants

This study is a follow-up study of the FRactionated heparin in women with Utero-placental Insufficiency and Thrombophilia (FRUIT-RCT).[23] The FRUIT-RCT included 139 women in the Netherlands (n=126), Sweden (n=3) and Australia (n=10) in the period between January 2000 and December 2009. Inclusion criteria were inheritable thrombophilia and a history of placental insufficiency: HD and/or a small-for-gestational age (SGA) infant, and a delivery

before 34 weeks gestation. HD was defined as preeclampsia, eclampsia or HELLP-syndrome. Subjects were randomized to receive either both daily low-molecular-weight heparin (LMWH, Dalteparin, weight adjusted) and daily aspirin, or daily aspirin only.

For the current follow-up study we have invited women who lived in the Netherlands with HD during the FRUIT-RCT and matched them with women without HD for age, study arm and chronic hypertension at start of the FRUIT-RCT. We excluded women with SGA without HD in the FRUIT-RCT index pregnancy. We visited all women in one of the nearest hospital in the area they lived. The study was approved by the medical ethical committee of the VU University Medical Center in Amsterdam. All women participating in the study provided written informed consent.

Measurements

A validated questionnaire was used which included questions about medical history, current use of medication and family history including cardiovascular disease.[24]

Physical examination included blood pressure measurements which was measured manually with a validated sphygmomanometer in sitting position at the right upper arm. The mean of two measurements was used in the analyses. Physical examination also included height, weight, hip and waist circumference measurements.

Venous blood samples were collected after an overnight fast and assayed for glucose, insulin, total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, creatinine, ureum and high-sensitivity c-reactive protein (hs-CRP). Insulin resistance was assessed by the homeostatic model assessment (HOMA): insulin concentration/ (22.5 e^{-ln} glucose concentration). Urine was collected for assessment of microalbuminuria and creatinine immediately after waking up to calculate the albumin creatinine ratio. After the blood and urine collection, the samples were taken to the laboratory of the VU University Medical Center and analysed within five hours after blood draw.

Definition of clinical diagnosis

Hypertension was defined as a systolic blood pressure ≥140 mmHg, a diastolic blood pressure ≥ 90 mmHg or current use of antihypertensive medication. Presence of diabetes mellitus (DM) was defined as fasting glucose ≥ 7.0 mmol/l or treatment (diet or medication). Hypercholesterolemia was defined as total cholesterol ≥5.0 mmol/l or current use of statins. Increased cardiovascular risk as composite outcome is defined as presence of either hypertension, hypercholesterolemia, BMI>25 or DM. Metabolic syndrome was defined according to the National Cholesterol Education Program (NCEP) Adult treatment Panel III (ATPIII panel) which means presence of three or more of the following: fasting plasma glucose ≥ 6.1 mmol/l, serum triglycerides ≥ 1.69 mmol/l, serum HDL-cholesterol ≤ 1.29 mmol/l, blood pressure ≥130/≥85 mmHg or waist circumference ≥ 88 cm.[25]

Statistics

Differences in baseline characteristics of the study population at screening visit were examined using an Independent T-test or a Fisher's Exact Test when appropriate. Obstetric history in

the FRUIT-RCT and cardiovascular risk factors were examined using a Fisher's Exact Test, Chi-2 test, Independent T-test or Mann-Whitney U test when appropriate. Means and standard deviation (SD), numbers and percentages or median and interquartile range (IQR) were reported. Statistical analyses were performed with IBM SPSS version 22.0 (SPSS Inc, Chicago, USA). A two-tailed *p*-value <0.05 is considered to be significant.

Results

In total, 41 women met the in- and exclusion criteria for this follow-up study and were invited. Out of these 41 women, two deceased (one due to a cerebral lymphoma, the other due to cerebral aneurysm), five were lost to follow-up and 12 refused participation resulting in 22 participants. Of the 22 included women, 11 women developed recurrent HD during the FRUIT-RCT and the other 11 women did not develop recurrent HD during the FRUIT-RCT. The year of index pregnancy (in which all women had early-onset HD) varied from 1996 until 2007; 8-19 years before the current follow-up study.
Characteristics of the women at study visit including items of the questionnaire are presented in Table 1. No differences between groups were demonstrated.
Obstetric history of both index and study pregnancy are given in Table 2. Of the 11 women with recurrent HD, four women had recurrent early-onset HD.

Table 1: Characteristics of study population at screening visit.

	Recurrent HD (n=11)	**Single HD** (n=11)	***p-value***
Age (years)	44.5 ± 3.6	44.7 ± 4.2	0.91
Non-caucasian	1 (9.1)	0 (0)	1.00
Years since delivery in FRUIT-RCT	9.1 ± 2.7	10.0 ± 3.3	0.49
Current smoker	0 (0)	2 (18.2)	0.48
Family history of vascular disease^			
First degree family (parents or siblings): arterial	3/10 (30)	3 (27.3)	1.00
First degree family (parents or siblings): venous	2/10 (20.0)	4/10 (40)	0.81
Mother and/or sister HD	6 (54.5)	2 (18.2)	0.18
Thrombophilia disorder*			
Protein S deficiency	1 (9.1)	1 (9.1)	1.00
Protein C deficiency	3 (27.3)	1 (9.1)	0.59
Factor V Leiden	8 (72.7)	5 (45.5)	0.39
Prothrombin gene G20210A mutation	1 (9.1)	4 (36.4)	0.31

Data are depicted ad mean ± SD or numbers (%).
HD: hypertensive disorders of pregnancy
^ some women did not know their family history, *some women were diagnosed with more than one thrombophilia disorder, thrombophilia was diagnosed before inclusion of the FRUIT-RCT and >10 weeks after (index) pregnancy.

Table 2: Obstetric history in women with recurrent and single HD in index and study pregnancy of the FRUIT-RCT

	Recurrent HD (n=11)	**Single HD** (n=11)	***p-value***
Index pregnancy			
SGA infant index pregnancy	5 (45.5)	5 (45.5)	1.00
GA delivery index pregnancy (days)	207 [196 – 219]	205 [197 – 219]	1.00
Study pregnancy			
Chronic hypertension at start pregnancy	4 (36.4)	3 (27.3)	1.00
Treatment with LMWH and aspirin	7 (63.6)	6 (54.5)	1.00
Treatment with aspirin only	4 (36.4)	5 (45.5)	1.00
SGA infant study pregnancy	2 (18.2)	2 (18.2)	1.00
GA delivery study pregnancy (days)	261 [238 – 276]	276 [260 – 281]	0.24

Data are depicted as mean ± SD, numbers (%) or median [IQR].
HD: hypertensive disorders of pregnancy, LMWH: low-molecular-weight heparin, SGA: small-for-gestational age, GA: gestational age.

Physical examination showed that women with recurrent HD had a higher systolic and diastolic blood pressure than women with single HD (Table 3a). Out of seven women with chronic hypertension at start of the FRUIT-RCT, five still had hypertension during the follow-up visit. The other two women (both in the recurrent HD group) did not take antihypertensive medication and had a normal blood pressure at follow-up.
Biochemical results showed a difference in the albumin creatinine ratio (Table 3b).
The composite outcomes increased cardiovascular risk as well as metabolic syndrome were found to be equally high in both groups (Table 3c).

Discussion

In this follow-up study almost three-quarter of women with inheritable thrombophilia and either single or recurrent HD had an increased cardiovascular risk, 8-19 years after early-onset HD. Systolic blood pressure, diastolic blood pressure and albumin creatinine ratio was higher in women with recurrent HD compared to women with single HD. Overall, nearly half of the women had hypertension, a third metabolic syndrome and a third hypercholesterolemia. The high prevalence of hypertension and hypercholesterolemia after HD in these women with inheritable thrombophilia is in line with other studies.[8;26] The prevalence of metabolic syndrome after HD is higher in the present study compared to other studies where they describe prevalences between 14-25%.[8;27] The difference of our study compared to the above mentioned studies is that women who participated in our study all had inheritable thrombophilia, were on average ten years older at follow-up and were examined after a longer time interval since pregnancy: the other studies examined women 0.5-2.5 years after pregnancy.[8;26;27] Moreover, two of three studies did not include early-onset HD but

Table 3a-c: Cardiovascular risk factors after recurrent and single HD: a) physical examination, b) biochemical results, c) clinical diagnoses Table 2: Obstetric history in women with recurrent and single HD in index and study pregnancy of the FRUIT-RCT

	Recurrent HD (n=11)	**Single HD** (n=11)	**Total group** (n=22)	***p-value***	**RR (CI)**
Table 3a: Cardiovascular risk factors at follow-up, physical examination					
Body composition					
Body mass index (kg/m^2)	27.0 ± 5.1	25.7 ± 4.3	26.3 ± 4.7	0.52	NA
Waist circumference (cm)	84.5 ± 11.5	84.9 ± 11.0	84.7 ± 11.0	0.93	NA
Hip circumference (cm)	107.5 ± 9.8	107.0 ± 8.0	107.2 ± 8.7	0.91	NA
Waist-to-hip ratio	0.78 ± 0.1	0.79 ± 0.1	0.79 ± 0.1	0.79	NA
Blood pressure					
Antihypertensive medication use	3 (27.3)	1 (9.1)	4 (18.2)	0.59	1.7 (0.8-3.6)
Systolic blood pressure (mm Hg)	133.2 ± 16.5	117.8 ± 17.4	125.5 ± 18.3	0.046	NA
Diastolic blood pressure (mm Hg)	87.7 ± 6.1	78.1 ± 11.7	82.9 ± 10.4	0.029	NA

	Recurrent HD (n=11)	**Single HD** (n=11)	**Total group** (n=22)	*p-value*	RR (CI)
Table 3b: Cardiovascular risk factors at follow-up, biochemical results					
Lipids					
Total cholesterol mmol/L	4.81 ± 0.68	5.06 ± 1.22	4.94 ± 0.97	0.55	NA
HDL mmol/L	1.48 ± 0.39	1.55 ± 0.38	1.52 ± 0.38	0.67	NA
Triglycerides mmol/L	1.25 ± 0.50	1.17 ± 0.80	1.21 ± 0.65	0.80	NA
Glucose metabolism					NA
Fasting blood glucose mmol/L	5.36 ± 0.44	5.29 ± 0.43	5.33 ± 0.43	0.70	NA
Insulin pmol/L	67.82 ± 24.63	82.27 ± 83.11	75.05 ± 11.92	0.59	NA
HOMA score	16.36 ± 6.75	20.35 ± 22.3	18.35 ± 16.21	0.58	NA
Inflammation					NA
hs-CRP mg/L	1.21 [0.51-3.83]	0.95 [0.46-2.03]	0.97 [0.50-2.30]	0.56	NA
Renal function					NA
Creatinine µmol/l	75.82 ± 15.73	75.36 ± 7.13	75.59 ± 60.27	0.93	NA
eGFR ml/min/1.73m	88.0 [70.0-90.0]	86.0 [76.0-89.0]	86.5 [75.5-90.0]	0.65	NA
Ureum mmol/L	4.91 ± 1.13	5.15 ± 1.19	5.03 ± 1.14	0.64	NA
Albumin creatinine ratio	1.06 [0.66-4.80]	0.37 [0.27-0.56]	0.58 [0.32-1.13]	0.008	NA

	Recurrent HD (n=11)	Single HD (n=11)	Total group (n=22)	*p-value*	RR (CI)
Table 3c: Cardiovascular risk factors at follow-up, clinical diagnoses					
Hypertension	7 (63.6)	3 (27.3)	10 (45.5)	0.09	2.1 (0.9-5.2)
Hypercholesterolemia	3 (27.3)	5 (45.5)	8 (36.4)	0.66	0.7 (0.2-1.8)
Body mass index > 25 (kg/m^2)	6 (54.5)	5 (45.5)	11 (50)	0.67	1.2 (0.5-2.8)
Diabetes Mellitus	0 (0)	0 (0)	0 (0)	-	NA
Increased cardiovascular risk	8 (72.7)	8 (72.7)	16 (72.7)	1.00	1.0 (0.4-2.6)
Metabolic syndrome	4 (36.4)	3 (27.3)	7 (31.8)	1.00	1.2 (0.5-2.8)

Data are depicted as mean ± SD or numbers (%).
HD: hypertensive disorders of pregnancy, RR: Relative Risk, CI: confidence interval, NA: not applicable, hs-CRP: high-sensitivity c-reactive protein, eGFR: estimated Glomerular Filtration Rate.
Increased cardiovascular risk is defined as presence of either hypertension, hypercholesterolemia, BMI>25 or DM.

late-onset HD only.[26;27] The other study included women with either early-onset HD, late-onset HD or pregnancy induced hypertension.[8] These three studies excluded patients with chronic hypertension prior to pregnancy, whereas 31.8% of the population in the present study had chronic hypertension at start of the FRUIT-RCT pregnancy. A fourth study was more comparable to our study, in which they examined women with the same history of early-onset HD as well as ten years after pregnancy.28 They found the same prevalence of hypertension (43.1%) and hypercholesterolemia (38.6%), however, a lower prevalence of metabolic syndrome (18.0%) compared to our study (45.5%, 36.4% and 31.8% respectively). Difference to our study is their age at follow-up; on average about five years younger compared to our population.[28] Since we found equal prevalences of hypertension and hypercholesterolemia and a higher prevalence of metabolic syndrome compared to women with unknown thrombophilia status,[8;26-28] we could not support the finding that thrombophilia might mediate in lowering cardiovascular risk factors compared to women without thrombophilia.[22] Moreover, the prevalences in the general Dutch female population in the age range of 40-49 year for hypertension (15%), hypercholesterolemia (17%) and metabolic syndrome (11%) are much lower compared to this study.[29]

The differences in systolic blood pressure, diastolic blood pressure and albumin creatinine ratio in women with recurrent HD is in line with the theory emerging of the results of retrospective studies, suggesting that women with a history of recurrent HD have a higher risk to develop CVD later in life compared to women with a history of single HD.[9;10] A systematic review, based on the same retrospective studies states that recurrent preeclampsia is associated with a sevenfold increased risk of later hypertension compared to a single episode.[2] We could not demonstrate a significant increase in the diagnosis hypertension in women with recurrent HD compared to women with single HD, but did demonstrate an increased systolic and diastolic blood pressure. Besides a higher albumin creatinine ratio in women with recurrent

HD, none of the other parameters differed between both groups. A possible explanation is that women with a history of early-onset HD already have such a high risk profile, that recurrence of the disease does not elicit a further increase. The previous mentioned studies had a retrospective set-up, which makes the diagnosis of HD more uncertain compared to our study with a prospective set-up.[9,10] Secondly, the endpoint of the study of Wikström et al was ischaemic heart disease, whereas we report on cardiovascular risk factors.[10]
Our results show a high prevalence of hypertension, metabolic syndrome and hypercholesterolemia in women with inheritable thrombophilia which is comparable with other studies examining women with a history of HD in whom the thrombophilia status is unknown. The results of our study suggest that presence of inheritable thrombophilia does not play a role in a further increase of the development of cardiovascular risk factors. This could be explained by the high risk due to the history of early-onset HD. A next step should be an individualized patient data meta-analysis to examine the influence of thrombophilia on cardiovascular risk factors in women with a history of early onset HD.[8;28]
Strength of this study is that to our knowledge this is the first study which examined cardiovascular risk factors in women with inheritable thrombophilia and a history of either single and recurrent HD in a prospective setting. Limitation of this study is the small population. Larger prospective studies are needed to examine if women with a history of recurrent HD have more cardiovascular risk factors compared to women with a history of single HD. In the composite outcome, increased cardiovascular risk, the definition of elevated BMI is arbitrarily chosen based on the demonstrated relation between a BMI >25 and CVD mortality.[30]

Conclusion

In this follow-up study of the FRUIT-RCT, nearly three-quarters of the women had an increased cardiovascular risk, irrespective of single or recurrent HD. Women with recurrent HD did have higher systolic and diastolic blood pressure and albumin creatinine ratio. This is similar to other studies examining cardiovascular risk factors in women with a history of HD without knowledge about their thrombophilia status.

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Hypertensive disorders of pregnancy appear not to be associated with Alzheimer's disease later in life

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Abstract

Background: The pathophysiological effects of hypertensive disorders of pregnancy on cerebrovascular function are supposed to be reversible. In contrast, higher rates of subjective cognitive complaints including memory, attention and concentration deficits are reported years after pregnancies complicated by hypertensive disorders. Moreover, the prevalence of white matter lesions is more prevalent after pregnancies complicated by hypertensive disorders.
It is well recognized that subjective cognitive complaints and white matter lesions are related to the occurrence of Alzheimer's disease (AD). Therefore, we hypothesized that women who have had a pregnancy complicated by hypertensive disorders are at increased risk of AD later in life.

Methods: We performed a nested cohort study for which 251 women from the Alzheimer Centre of the VU University Medical Centre in Amsterdam with AD and 249 women without AD were identified. We performed paper surveys and telephone surveys to assess the history of hypertensive disorders of pregnancy. Cerebral MRIs of women with AD and a reported history of hypertensive disorders of pregnancy were examined for presence of white matter lesions using the Fazekas score.

Results: The overall response rate was 85.2%. We found no significant difference between women with and without AD reporting a pregnancy complicated by a hypertensive disorder (12,7 vs 25.9% respectively P=0.11). Analysing hypertensive disorders in detail; no association between AD and preeclampsia was found (2.9% in women with AD versus 3.1% in women without AD, P=0.62). In total, four women with AD reported a preeclampsia in history. MRIs of these four women with AD showed that one woman had a Fazekas score of 1; the other three women had Fazekas scores of 0.

Conclusion: A reported history of hypertensive disorders in pregnancy appears not to be associated with Alzheimer's disease later in life. In addition, we found no association between preeclampsia per se and AD. These findings suggest (at least partly) different pathophysiological pathways of cerebrovascular damage associated with hypertensive disorder of pregnancy and those related to AD. This is in line with the well described heterogeneous syndrome of hypertensive disorders of pregnancy, its related wide spectrum of clinical symptoms and associated wide spectrum of involvement of cerebrovascular damage.

Introduction

Hypertensive disorders in pregnancy are a major cause of maternal and fetal morbidity and mortality.[1-4] Hypertensive disorders in pregnancy are the most common disorders in pregnancy; its prevalence is almost 10%.[2,5,6] These disorders include preeclampsia (blood pressure systolic ≥140mm Hg and/or diastolic ≥ 90mm Hg and proteinuria), pregnancy induced hypertension (hypertension as defined in preeclampsia, without proteinuria) and eclampsia. Despite extensive research, the exact causes of hypertensive disorders in pregnancy are still unknown. The main hypothesis is related to disturbed placental function in early pregnancy.[7] As a consequence generalised endothelial dysfunction develops, through which preeclampsia potentially affects the perfusion of several organs including liver, kidneys and brain.[7]
Until recently, the pathophysiological and clinical effects of hypertensive disorders in pregnancy were thought to be reversible including cardiovascular and cerebrovascular function. However, increasingly more evidence points to the fact that hypertensive disorders in pregnancy are associated with long-term effects on women's health, e.g. increased risk of cardiovascular and cerebrovascular disease.[8-21]

Long-term effects on cerebrovascular disease after a pregnancy complicated by (pre) eclampsia have been observed including a higher prevalence of subjective cognitive complaints including memory, attention and concentration deficits after several years, compared to women with a history of uncomplicated pregnancies.[15-18] Neuro-imaging studies show the evidence of long-term effects of (pre)eclampsia on the brain, including more frequently and lager cerebral white matter lesions compared to women who have had an uncomplicated pregnancy.[19-21]

The clinical implications of the presence and larger volume of white matter lesions in relatively young women remains unclear.[17,22] In elderly patients white matter lesions have been described and linked with subjective cognitive complaints and Alzheimer's disease (AD) pathology.[23-28] Subjective cognitive complaints can be the first sign of AD in elderly whose objective cognitive performance is normal.[29] Furthermore, white matter lesions on cerebral magnetic resonance imaging (MRI) correlate with subjective cognitive failure,[30] and the severity of white matter lesions is shown to predict the rate of cognitive decline over time.[31,32] These white matter lesions are suggested to play an important role in the pathogenesis of dementia.[33-35] Although the aetiology of AD is still not completely elucidated, it is now known that the presence of cerebral white matter lesions, vascular risk factors and endothelial dysfunction contribute to its development.[36-39]
Based on the above we hypothesize that women who have had a pregnancy complicated by hypertensive disorders are at increased risk of AD in later life.

Materials and methods

Our study was a retrospective nested cohort study, in which we compared the reported prevalence of hypertensive disorders of pregnancy between women with AD and women without AD.

Participants

We identified women from the database of the Alzheimer Centre of the VU University Medical Centre in Amsterdam with AD. All women had given written consent to participate in scientific research previously. For the control group, we selected partners of male AD patients who gave their consent for participating in scientific research.

Participants were included when they had at least one pregnancy continuing after 24 weeks gestation. Women were excluded when they had a history of other types of neurodegenerative diseases, thromboembolisms, systemic lupus erythematosus or diabetes prior to their first pregnancy, or when nulliparous. Also women with other forms of dementia like vascular dementia and mild cognitive impairment were excluded. The medical ethical committee of the VU University Medical Centre approved the study.

Surveys

We used a self-report method to gather data concerning obstetric complications. Recall of hypertensive disorders in pregnancy is adequate, according to a systematic review which was conducted to comprehensively review and assess the available literature on maternal recall of hypertensive disorders in pregnancy, so we hypothesized that recall, even in AD patients, would be sufficiently reliable.[40]

Initially, we sent all our participants a paper survey for assessment. We used a questionnaire, including items about demographic background, lifestyle, medical history and obstetric history.[41] The obstetric history items included the number of pregnancies, parity, live births and stillbirths. Also items concerning obstetric complications; hypertension, low-salt diet, proteinuria, toxaemia (previous term for preeclampsia) or preeclampsia and seizures or eclampsia were included. Secondary to the initial paper survey, we performed a telephone survey in non-responders to achieve an optimal response rate and prevent response bias. In both paper and telephone surveys partners or family members were allowed to support the participants to help complete the surveys, to gather as much information as possible.

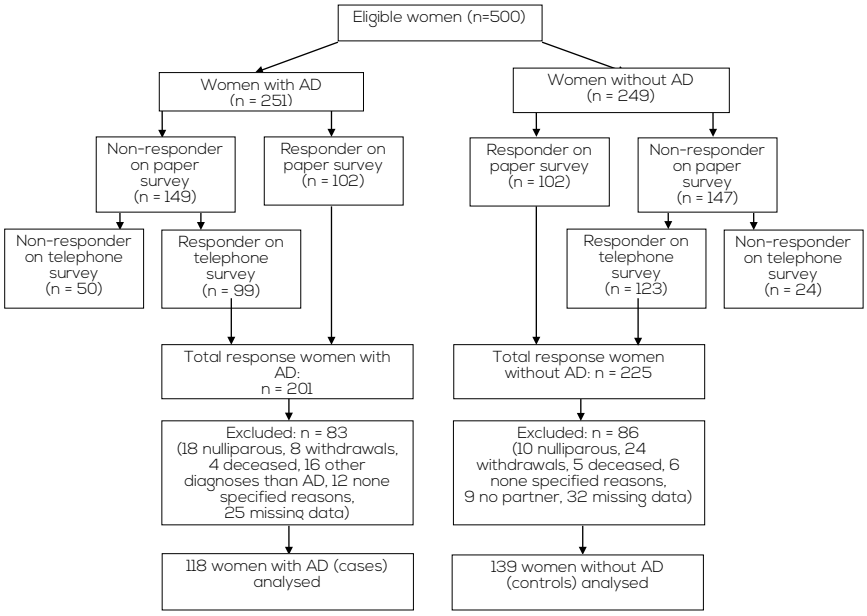
Cerebral MRI

In women with AD and a reported history of hypertensive disorders of pregnancy we performed file searches to investigate pathophysiological associations with both AD and hypertensive disorders of pregnancy, focussing mainly on the occurrence of white matter lesions in cerebral MRI-scans.

MRIs were acquired on a 3T whole body MR system (MR750, GE Medical Systems, Milwaukee, WI, USA; Ingenuity TF PET/MR, Philips Medical Systems, Best, The Netherlands; Titan, Toshiba Medical Systems, Japan). The standard MRI protocol included a sagittal 3D heavily

T1-weighted gradient-echo sequence with coronal reformats, a sagittal 3D T2-weighted fluid-attenuated inversion recovery (FLAIR) turbo/fast spin-echo with axial reformats, a transverse T2-weighted turbo/fast spinecho, a transverse T2* susceptibility sequence, and diffusion weighted imaging/EPI. All sequences were performed with whole brain coverage. An experienced neuroradiologist reviewed all scans. Temporal lobe atrophy (MTA) was scored from 0-4 (score 0: no atrophy; score 1: only widening of choroid fissure; score 2: also widening of temporal horn of lateral ventricle; score 3: moderate loss of hippocampal volume (decrease in height); score 4: severe volume loss of hippocampus). Global cortical atrophy (GCA) was scored from 0-3 (0: no cortical atrophy; 1 mild atrophy: opening of sulci; 2 moderate atrophy: volume loss of gyri; 3 severe (end-stage) atrophy: 'knife blade' atrophy). White matter hyper intensities were rated using the Fazekas score and classified as punctuate (grade 1), beginning confluent (grade 2), or confluent (grade 3) [30]. Number of micro bleeds were counted on T2* sequences and were defined as small round foci of hypo-intense signal, up to 10mm in brain parenchyma [31]. Numbers of infarctions were also counted.
Inter- and intrarater weighted kappas of at least 0.80 for MTA, 0.60 for GCA, and 0.70 for Fazekas (against internally established gold standard) were required.32 For microbleed count, weighted Cohen's kappa are >0.90.[31,32]

Figure 1: flow-chart. AD: Alzheimer's disease.



Sample size considerations and statistical analysis

In the general Dutch population, the prevalence of hypertensive disorders of pregnancy is 8.48% (Dutch Perinatal Registry). We expected to find a prevalence of hypertensive disorders of pregnancy twice as high in women with AD compared to women without AD. To achieve sufficient statistical power with an alpha of 0.05 and a beta of 0.84, a required minimum of

Table 1: Baseline characteristics. All items represent the status at time of survey.

	n	Women with AD (n=118)	n	Women without AD (n=139)	p
Age[1]	113	67.1 (10.6)	132	64.2 (10.3)	.035*
BMI[3]	108	23.3 (5.6)	134	24.5 (6.3)	.014*
Caucasian background[2]	117	108 (92.3)	130	119 (91.5)	.83
Smoking[2]	114	12 (10.5)	136	16 (11.8)	.76
Alcohol use >1 per day[2]	114	64 (56.1)	136	87 (64.0)	.21
Parity[1]	118	2.5 (1.0)	139	2.4 (0.9)	.69
Live births[1]	118	2.4 (0.9)	139	2.4 (0.9)	.76
Self-report	117	31 (26.5)	138	132 (95.7)	.000*
Diabetes[2]	110	6 (5.5)	133	12 (9.0)	.290
Hypercholesterolemia[2]	110	20 (18.2)	132	25 (18.9)	.88
Hypertension[2]	110	20 (18.2)	134	38 (28.4)	.063
Myocardial infacrtion[4]	111	5 (2.7)	134	1 (0.7)	.094
Cerebrovascular accident[2]	112	3 (2.7)	135	8 (5.9)	.35
Renal disease[4]	110	2 (1.8)	133	7 (5.3)	.19
Thrombosis[4]	110	3 (2.7)	133	4 (3.0)	1.00
Pulmonary embolism[4]	109	1 (0.9)	134	2 (1.5)	1.00
Brain surgery[4]	111	0	135	1 (0.7)	1.00

BMI: Body Mass Index, AD: Alzheimer's disease
* Statistically significance (α = .05)
1. Mean and standard deviation; independent samples T-test
2. Number and percentage; Chi-square
3. Median and interquartile range; Mann-Whitney test
4. Number and percentage; Fisher's exact test

BMI: Body Mass Index, AD: Alzheimer's disease
* Statistically significance (β= .05)
1. Mean and standard deviation; independent samples T-test
2. Number and percentage; Chi-square
3. Median and interquartile range; Mann-Whitney test
4. Number and percentage; Fisher's exact test

233 participants per group was needed (tested double-sided).

To analyse baseline characteristics the parametric student's t-test, chi-square test, non-parametric Mann-Whitney U test or Fisher's exact tests were used.

Chi-square test was used to compare presence of a reported history of hypertensive disorder in pregnancy, preeclampsia, eclampsia or pregnancy induced hypertension between women with and without AD. When appropriate, we conducted multiple logistic regression analyses to correct for potential confounders. Statistical analyses were performed with IBM SPSS 22.0 (SPSS Inc., Chicago, IL). Results were considered significant at the 5% level.

Results

In total, 500 women were identified from the database of the Alzheimer Centre of the VU University Medical Centre; 251 women with AD and 249 women with a partner with AD. The overall response rate was 85.2%, 201 women with AD and 225 women without AD, consisting of 102 women responding to paper surveys in each group (40.6% and 40.9% respectively), and 99 (39.4%) women with AD and 123 (49.4%) women without AD responding to telephone survey. Of these 426 women, we excluded 169 women; 28 due to nulliparity, 16 women due to diagnoses of dementia different from AD (e.g. vascular dementia or mild cognitive

Table 2: Incidence of hypertensive disorders of pregnancy.

	Women with AD		Women without AD		
	n	n (%)	n	n (%)	p
Pregnancy induced hypertension (PIH)	118	11 (9.3)	139	32 (23.0)	.031*
Preeclampsia	118	4 (3.7)	139	4 (2.9)	1.00*
Eclampsia		0		0	
Hypertensive disorders of pregnancy	118	15 (12.7)	139	36 (25.9)	.11*

AD: Alzheimer's disease
Number and percentage
* after correction for age and body mass index.

Table 3: Results of MRI of four women with Alzheimer's disease and a history of preeclampsia.

preeclampsia.

	Patient 1	Patient 2	Patient 3	Patient 4
MTA left hemisphere	1	2	0	0
MTA right hemisphere	0	2	0	1
GCA	1	0	1	0
Fazekas score	0	0	0	1
Micro bleeds	No	No	No	No
Infarcts	No	No	No	No

MTA: Medial temporal lobe atrophy (score 0-4)
GCA: Global Cortical Atrophy (score 0-3)
Fazekas score (score 0-3)

impairment), 32 women were not willing to participate in the study (e.g. women could not answer the questions themselves anymore, carers did not have the time for it), 9 women have been deceased, 57 participants had essential missing data e.g. incomplete responses, 9 men had no (female) partner and finally 18 women were non-responders due to other reasons. As described in Figure 1, a total of 257 women were analysed, 118 women with AD and 139 without. Of the women with AD, 31 (26.5%) answered the survey themselves; of the women without AD, 132 (95.7%) did. In two paper surveys (one woman with and one without AD) the reporter was unclear.

The baseline characteristics are depicted in. Women with AD were significantly older (P=0.035) and had lower Body Mass Indices (BMI) when they filled in the survey than women without AD (P=0.014). Three women (all three had AD) reported both preeclampsia and pregnancy induced hypertension in their history. In these cases, we classified these women with a history of preeclampsia and not with pregnancy induced hypertension, to prevent overestimation of hypertensive disorders of pregnancy in this group.

AD in relation to hypertensive disorders of pregnancy

There was no difference in the number of women with AD reporting a pregnancy complicated by hypertensive disorders than women without AD (12.7% versus 25.9%, P=0.107 after correction for maternal age and BMI, Table 2). We detected no significant difference in a

reported history of preeclampsia between women diagnosed with AD and those without AD (3.7% versus 2.9%, P= 1.000 after correction for age and BMI, Table 2). In both groups none of the women reported a history of eclampsia.
Women with AD less often reported pregnancy induced hypertension, than women without AD (9.3% vs 23.0%, P=0.031 after correction for age and BMI).
We evaluated whether self-reports differed from reports by partners and family members. The reported prevalence of hypertensive disorders of pregnancy was comparable to the overall reported prevalence of hypertensive disorders of pregnancy for both self-reports (respectively 16.1% versus 25.8%) and reports by others (respectively 11.6% versus 33.3%).

Cerebral MRI
We examined the cerebral MRIs of the four women with AD and a reported history of preeclampsia. In Table 3 the results of the MRI are depicted. Three of the four women had a MTA score of 1 or 2 in either their left or the right hemisphere. The fourth woman had a score of 0. Two women had a GCA of 1; the other two women had a score of 0. The Fazekas score was 0 in three women, in the fourth woman, the Fazekas score was 1. In none of the women micro bleeds or infarctions were seen. All four women did have early-onset AD (<65 years at moment of diagnosis).

Discussion

We found no difference in reported history of hypertensive disorders in pregnancy between women with and without AD. This may suggest different pathophysiological pathways of cerebrovascular damage between AD and hypertensive disorders of pregnancy.
In a sub analysis, we found that women with AD reported preeclampsia rates comparable to women without AD. However, women with AD reported pregnancy induced hypertension less frequently in comparison to women without AD. Remarkably, in women without AD we encountered an incidence of pregnancy induced hypertension of 25.9%, far higher than in the normal population, since the prevalence of hypertensive disorders in pregnancy is almost 10% and pregnancy induced hypertension is only a part of this 10%.[2,5,6] A first explanation could be that hypertensive disorders of pregnancy is a heterogeneous syndrome, with a wide spectrum of clinical symptoms and differences in presentation. A second possible explanation for this finding could be over reporting due to recall bias. A systematic review showed that length of recall of hypertensive disorders of pregnancy did not appear to affect recall quality (up to 30 years), though sensitivity was lower and less consistent for pregnancy induced hypertension than for preeclampsia [40]. Also, the definition medics use for pregnancy induced hypertension (systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg measured on two occasions 6 hours apart[41]), could be stricter than the definition patients apply. Over reporting of pregnancy induced hypertension is a phenomenon described in other studies as well, however an explanation is lacking.[42,43] Furthermore, the difference in recall between groups could also be explained by the fact

that it might be possible that women with AD do not remember their obstetric history as well as women without AD. Although the survey we used was validated,[44,45] it is not validated for this specific population in women with AD. It is possible that women with AD underestimated their incidence of hypertensive disorders of pregnancy and therefore the recall bias could affect both groups; overestimation in the controls and underestimation in the women with AD. Moreover, not all women answered the survey themselves, especially in the group with women with AD. We interviewed several partners or other family members and we included only those who remembered sufficient details about the pregnancy and delivery. Unknown is the difference in recall bias between women themselves, or their partners or other family members.

The clinical implications of cerebral white matter lesions in young women with a history of preeclampsia and eclampsia are unknown, as is the exact cause and consequence in the long term of previously found cognitive complaints in these women. To our knowledge there are only two studies that report a link between preeclampsia and AD.[46,47] The data of the first study indicate that the STOX1 gene controls a conserved pathway shared between placenta and brain with overexpression in late-onset AD (>65 years at time of diagnosis). A difference with our study is that the women with AD who reported preeclampsia, all had early-onset AD.46 In the second study they found that women with preeclampsia exhibit urine congophilia where as healthy pregnant women did not .[47] This is a marker of protein instability and misfolding and has been used as a post-mortem histological indicator of Alzheimer's β-amyloid deposits in the brain of AD patients.[48,49]

The number of women with AD and a history of preeclampsia was low and therefore the number of MRIs we could examine was too low to draw any conclusion. In comparison to the studies mentioned afore, the timeframe between a hypertensive pregnancy and cerebral imaging is different.[19-21] These studies performed the MRI much earlier after pre(eclampsia) then we did i.e. on average seven years after pregnancy. On the other hand, in more advanced maternal age, more white matter lesions could be expected, but in our four examined MRIs, only one woman had white matter lesions. To our knowledge, no studies are performed to investigate white matter lesions after pregnancy induced hypertension.
Alternatively, the pathophysiological effects of hypertensive disorders of pregnancy on cerebrovascular function after all, is completely reversible, but that it might take a couple of decades for the brain to fully recover, since earlier studies did show a relation between pre(eclampsia) and white matter lesions on average seven years after pregnancy.[19-21]

Strength of our study is the novelty; to our knowledge, no other studies have been performed to investigate if patients with AD had higher prevalence of hypertensive disorders of pregnancy in their histories. Furthermore, we had a large response rate of 85.2%. Among the limitations of our study there is the design (a retrospective cohort study). Preferably we would perform a prospective study, but since it would take a couple of decades to be able to perform such a study, in our opinion, this was the best approach (this being an explorative study). Also, the number of patients with AD and a reported history of hypertensive disorders

of pregnancy were low; we need larger samples of these subjects to assess white matter lesions on MRI-level, to draw conclusions. Moreover, we did not achieve our power criteria; based on a prevalence of hypertensive disorders of pregnancy of 8.48% according to the Dutch Perinatal Registry, we aimed to include 233 women per group, we were able to include only 118 and 139 women in each group.
A power analysis for future research is performed for a relationship between AD and preeclampsia using the results of our study. Since 3.1% of the women with AD had preeclampsia and 2.9% of the women without AD had preeclampsia, with an alpha of 0.05, a power of 0.8, tested 2-sided, 114.068 women per group are needed for a follow-up study. This number represents women with a history of hypertensive disorders of pregnancy (including pregnancy induced hypertension).

Conclusion

A reported history of hypertensive disorders in pregnancy appears not to be associated with Alzheimer's disease later in life. In addition, we found no association between preeclampsia and AD in our study. Women with AD did report less often pregnancy induced hypertension, than women without pregnancy induced hypertension, possibly due to recall bias and a lack of power. Furthermore, we could not support earlier studies that showed an association between white matter lesions and hypertensive disorders of pregnancy, also since the number of women with AD and a history of hypertensive disorders of pregnancy was low. Our findings suggest different pathophysiological pathways of cerebrovascular damage after hypertensive disorder of pregnancy and AD.

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General Discussion

General discussion

In this thesis, studies about anticoagulant and antiplatelet treatment strategies in relation to hypertensive disorders of pregnancy (HD) and long-term risks including cardiovascular risk factors and Alzheimer's disease after HD are presented. In the introduction of the thesis three main questions were raised:

1. Which populations benefit from low-molecular-weight heparin (LMWH) and aspirin during pregnancy and what is its effect on mother and fetus/neonate?
2. Do adherence for aspirin in pregnant women and aspirin resistance play a role in women with recurrent HD?
3. What are the cardiovascular risk factors in women with thrombophilia and a history of single or recurrent (early-onset) HD? Increases a history of HD the risk for Alzheimer's disease?

In this general discussion the three main questions will be discussed. Moreover, recommendations for clinical practice and implications for future research will be debated.

Populations that benefit from aspirin and LMWH during pregnancy and its effect on mother and fetus/neonate

The individualized patient data meta-analysis (IPDMA) aimed to investigate the effect of LMWH for the prevention of placenta mediated pregnancy complications, using randomized controlled trials (RCT) from various countries with and without beneficial effect. These RCT's included women with various general (e.g. with and without thrombophilia) and obstetric histories.[1-8] In the IPDMA, women were included with previous late pregnancy loss, small-for-gestational age (SGA) infants, placental abruption and HD. The IPDMA, only found a beneficial effect of LMWH in women with previous placental abruption (chapter 5).The conclusion of this IPDMA is that LMWH is not effective in the prevention of recurrent obstetrical complications irrespective of gestational age and severity of the complication. However, the major limitation of the IPDMA is the use of composite inclusion criteria and composite outcome, which makes tailored medicine not feasible. It is possible that in specific populations, a beneficial effect of LMWH exists.[1;3;9] The database of the IPDMA, has the opportunity to examine the effect of LMWH in specific populations (e.g. women specific family history; Low-molecular-weight heparin and the prevention of recurrent hypertensive disorders of pregnancy in women with inheritable thrombophilia and a family history of vascular disease; a confirmation and an unexpected finding, van Hoorn et al. submitted) or with specific obstetric history (e.g. preterm birth). Furthermore, the collaboration of this international group of researchers with different areas of expertise will give the opportunity to mobilize large number of patients for future projects.

The effect of LMWH and/or aspirin usage is examined in one specific population in this thesis: women with systemic lupus erythematosus (SLE) and/or primary antiphospholipid syndrome (APS) (chapter 3). Women with SLE without antiphospholipid antibodies (aPL) not treated with aspirin and/or LMWH had the best pregnancy outcomes compared to women treated

with aspirin and/or LMWH. However, the complications rate was still considerable with a preterm birth rate of 33.2% and intrauterine fetal death (IUFD) rate of 4.8%. A selection bias probably plays a role: physicians did not prescribe LMWH and/or aspirin in cases with a perceived low a priori risk for complications, concerning both obstetrical and SLE and/or APS history. On the other hand, it is noteworthy that most pregnancy complications occurred in women treated with LMWH and/or aspirin. Unfortunately, the number of women in the other three patient groups (SLE with aPL, SLE with APS and primary APS) were too low to perform statistical analyses on the effect of aspirin and/or LMWH.
Apparently, doctors prescribe LMWH to their pregnant SLE patients, without proven beneficial effect by means of a RCT or well documented case control studies. Side-effects of LMWH should be explained to the women, like possible bleedings and allergic reactions. It has been described that approximately 2% of the pregnancies with daily LMWH injections are complicated by bleedings either antepartum or postpartum.[10] However, another study and the IPDMA did not demonstrate more blood loss during delivery in women with LMWH compared to women without LMWH.[9,11] Early recognition of allergic reactions give the opportunity to change medications which is most often sufficient. A RCT which examines the effect of LMWH in women with SLE is currently lacking and would be of additional value.
Until now, there seems no role for LMWH in the prevention of second and third trimester complications in women with APS based on placenta mediated complications in the history as demonstrated in two RCTs. The first RCT examined the effect of LMWH on recurrent placenta mediated complications in women with a history of either recurrent placenta mediated complications irrespective of gestational age or venous thromboembolism or both.[4] The second RCT was performed in women with a history of HD or SGA infants and delivery before 34 weeks only.[12] LMWH should be considered in women with a history of thrombosis, for the prevention of venous thrombosis in pregnancy and the postpartum period.[10]

With the present knowledge there seems to be no indication for LMWH in women with SLE without antiphospholipid antibodies, and tailored medicine is required in case of SLE with aPL, SLE with APS and primary APS. Aspirin should be considered in all women with SLE and primary APS due to limited side-effects and proven beneficial effects in high-risk populations, although the exact working mechanism is unknown and a RCT is absent.13-16

The counselling of women with SLE and or APS can be extended with another finding of this cohort of women with SLE: pregnancy complications including HD, preterm birth, IUFD and SGA infants in consecutive pregnancies are as numerous as in the first pregnancy (chapter 2). This finding is not in line with observations in the general population, where multiparity reduces this risk, probably due to improvement of maternal-fetal immune adaptation in subsequent pregnancies.[17] Moreover, the first pregnancy appears to be predictive for the outcome in consecutive pregnancies, since almost half of the patients had no severe complication during all of their pregnancies and more than 40% developed a severe complication during all of their pregnancies. It would be interesting to identify which women are at risk. Unfortunately, we could not examine this in our cohort since a multivariate analysis investigating multiple risk

factors for pregnancy complications requires a larger cohort. Therefore, a large, preferably prospective, cohort study is needed. The preliminary results of our studies, however, can be used during preconceptional counselling. With the present knowledge, the Dutch obstetric guidelines concerning SLE and APS of the NVOG, Dutch society of Obstetrics and Gynaecology, are both from 2007 and can be updated.[18,19]

In a specific population of women with HD or SGA and delivery before 34 weeks gestational age in their obstetric history and inheritable thrombophilia, we found that the addition of LMWH to aspirin did not influence either fetal growth nor Doppler flow velocities in uterine and umbilical arteries (chapter 1). One should take into account that the RCT was powered to examine the additional effect of LMWH when added to aspirin for the primary outcome HD. In this population, measuring Doppler flow velocities was not an optimal tool to predict adverse pregnancy outcome. During preconceptional counselling, women should be told that they do have a risk for HD in consecutive pregnancies of around 20%, the risk for a SGA infant is considerable, about 30%, and the Doppler flow velocities are abnormal in almost 50%, reflecting a high risk of suboptimal placentation in these women.[1] In the IPDMA of van Oostwaard et al. recurrence rate of preeclampsia was 16.0%, irrespective of thrombophilia.[20]

Aspirin adherence and aspirin resistance

Adherence for aspirin during pregnancy cannot be taken for granted. Only two third was adherent to aspirin, depending on which questionnaire was used (chapter 6). One should take into account that the questionnaires we used, were quite strict. For instance the simplified medical adherence questionnaire defined non-adherence as more than two doses missed during the past week or in the past three months.[21] In advance, we expected to find higher adherence rates in our study, since we thought that women would be highly motivated to use aspirin daily, to prevent complications during pregnancy. We do realize that a limitation of our study was the small population, but all women had a similar indication for aspirin usage and thus the same motivation to reduce recurrent obstetrical complications. The results of our study will increase the awareness of the doctor to pay attention to adherence for aspirin during all regular check-ups and to support their patients. All patients should be informed about the importance of aspirin and its working mechanism. Aspirin is thought to improve the trophoblastic invasion of the uterine spiral arteries and might subsequently improve the development and efficacy of the placenta probably due to thrombocyte aggregation inhibition and/or an anti-inflammatory working mechanism.[15,22] Hereby, it lowers the risk for recurrent pregnancy complications.[15,16] Women should be better instructed not only to take their aspirin daily, but also that intake in the evening optimize its effect, since lower blood pressures in the third trimester are described compared to intake in the morning.[23-26]

We are well aware that adherence for LMWH might be a problem as well. The doctor should pay attention and explain the risks and benefits at start and check the injection sites for hematoma's, swelling and allergic reactions every visit to optimize acceptance of the long period of subcutaneous injections.

Aspirin resistance could be an explanation for the recurrence of HD in women treated with

aspirin alone in the FRactionated heparin in women with Utero-placental Insufficiency and Thrombophilia randomized controlled trial (FRUIT-RCT), as Bujold et al. hypothesized.[27] In our study we examined this in the FRUIT-RCT population and could not demonstrate a relation between aspirin resistance and recurrence of HD (chapter 7). We were surprised about this finding, since the hypothesis of aspirin resistance as cause for occurrence of a disease seemed plausible. The hypothesis of Bujold et al. cannot be rejected yet, with the present knowledge. One reason is that our study had the limitation that it was performed in non-pregnant women, 6-16 years after the study pregnancy. Therefore, another study is currently ongoing: the RADAR study: Resistancy of Aspirin During and After pRegnancy. Aim of this study is to examine whether aspirin resistance changes per trimester during pregnancy and if it is related to aspirin resistance outside pregnancy (chapter 8). Depending on the results of the RADAR study, we might incorporate aspirin resistance measurements in a recently started RCT. This RCT investigates if aspirin is beneficial in the prevention of recurrent preterm birth in women who are randomized into aspirin or placebo (protocol submitted). It will give the opportunity to compare platelet activity between women with and without aspirin usage. Another interesting subject for a future study would be to investigate in a prospective setting if a relation with adverse pregnancy outcome and aspirin resistance exists. The RADAR study is not powered to answer this question. A second reason that we cannot reject the hypothesis of Bujold et al. yet, is that we should keep in mind that a perfect device to examine aspirin resistance has not been developed yet. This is confirmed by our study since no consistency in the results between the devices could been demonstrated in our study. Postponing research projects examining aspirin resistance until a better device has been developed, should be considered.

Cardiovascular risk factors in women with thrombophilia and a history of single or recurrent (early-onset) HD and Alzheimer's disease in relation to HD

It is a commonly accepted theory that pregnancy is a stress test which can identify women who have an elevated risk to develop cardiovascular disease (CVD) later in life.[28-32] It has not been elucidated yet if HD is a disease within the CVD spectrum or that HD itself is a risk factor for CVD later in life.[33;34] Women with thrombophilia and recurrent HD had a higher systolic blood pressure, diastolic blood pressure and albumin creatinine ratio 8-19 years after early-onset HD compared to women with a history of single HD (chapter 9). Our study confirmed the results of retrospective cross-sectional studies, showing that recurrent HD increases the risk to develop CVD later in life compared to single HD[35;36] When compared with other studies, women with inheritable thrombophilia have a comparable risk to develop cardiovascular risk factors after HD compared to women without inheritable thrombophilia, only metabolic syndrome occurred more frequently.[37-40] However, a direct comparison between women with and without inheritable thrombophilia 8-19 years after (early-onset) HD is to our knowledge currently lacking. Data of a similar population without thrombophilia is currently collected and will give the opportunity to examine the role of thrombophilia itself on the risk to develop cardiovascular risk factors after HD. We should discuss if a history of HD is comparable with diabetes mellitus and rheumatoid arthritis requesting earlier start of

antihypertensive drug treatment in the prevention of CVD.[41] A prospective cohort of women in which pregnancy outcome is carefully recorded would be interesting. Another suggestion would be to evaluate pregnancies in large cohorts in which CVD is examined as well, for example the Framingham Heart Study or the Longitudinal Aging Study Amsterdam (LASA). The second study in this thesis which examined long-term outcome after HD did not show a relation between HD and Alzheimer's disease (chapter 10). However, we might not have used the right population to examine this relation. In our study we examined whether the prevalence of HD differed in women with and without Alzheimer Disease. HD causes generalized endothelial dysfunction, through which it potentially affects the perfusion of several organs including the brain.[42] But it would be interesting to focus also on women with vascular dementia, because demented patients with a large vascular burden could be the population in which a relation with HD exists, due to similar aetiology. Still there is support for a vascular aetiology in Alzheimer's disease.[43;44] After publication of our study, two cohort studies were published. One study supported our finding and found no relation between HD and dementia, including Alzheimer's disease.[45] The second study concluded that HD is associated with worse performance on tests of processing speed and smaller brain volumes decades after HD.[46] Both studies used the same method as we did: they used a questionnaire and asked in retrospect whether a woman had HD during any of their pregnancies. The major limitations of these studies, including ours, is the potential recall bias. A prospective cohort study is to our knowledge still lacking.

Answers to the woman discussed in the outline of this thesis

The gynaecologist attempts to answer the questions of the woman who visited the outpatient clinic 6 weeks after the caesarean section. Her second child was born at 32 weeks gestation due to recurrent HD.

What are reasons why aspirin does not prevent recurrent HD in every women?

The exact reason is still unknown and various factors may play a role. We learned that not all women are adherent for aspirin during pregnancy. It should be emphasized to use aspirin daily and, to lower blood pressure in the third trimester, in the evening which is not widespread yet. Whether aspirin resistance plays a role in the recurrence of HD is still unknown, however, the results of the study in this thesis do not point into that direction. We should inform women that aspirin reduces the risk for recurrent HD, and thus not prevent recurrence completely.

Could LMWH be of additional value to prevent recurrent HD in absence of proven thrombophilia?

Currently, there is a single RCT with evidence to prescribe LMWH to this patient in a future pregnancy: Rey et al. did find a beneficial effect of LMWH in women without thrombophilia and adverse obstetric history.[3] Nevertheless, their population (n=116) was also used within the IPDMA concluding that LMWH does not prevent placenta mediated pregnancy complications.[9] The same holds true for women with inheritable thrombophilia and adverse obstetric history.[1]

However, the inclusion and exclusion criteria were composite in the IPDMA and thus not clustered for early-onset HD with or without thrombophilia. Therefore, we have to present our knowledge of individual RCT's and the IPDMA to the patient and make a shared decision.

What are the future cardio- and cerebrovascular health risks for this woman with a history of recurrent HD?

She has an increased risk to develop chronic hypertension compared to women without HD.[38;39;47] It seems that recurrent HD increases the risk to develop cardiovascular risk factors, but more research is needed.[35-37] The current multidisciplinary developed guidelines recommend to create a cardiovascular risk profile at the age of 50 for women who did experience HD, so this patient would also be advised to have a check-up at that age.[41;48;49] An interesting field for examination is whether earlier evaluation of cardiovascular risk profile and thereby earlier start of prevention will be beneficial in the reduction of CVD. With the present knowledge, we cannot answer the question if this patient has an increased risk for Alzheimer's disease or other dementias.

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Summary

Summary

In this thesis anticoagulant and antiplatelet treatment options during pregnancy are examined to prevent placenta mediated pregnancy complications, including hypertensive disorders of pregnancy (HD) in high risk populations. Hypertensive disorders of pregnancy is an umbrella term for pregnancy induced hypertension, preeclampsia, eclampsia and HELLP syndrome (Hemolysis, Elevated Liver enzymes and Low Platelets). The women we examined had an adverse obstetrical history of placenta mediated pregnancy complications and/or an underlying disease increasing the risk for pregnancy complications, like systemic lupus erythematodes. Moreover, cardiovascular risk factors in women with thrombophilia after HD is examined, as well as a relation between HD and Alzheimer's disease.

In the **Introduction**, risk factors, preventive strategies, short- and long-term outcomes of HD and the outline of this thesis are described.

In **Chapter 1** we describe secondary outcomes of a randomized controlled trial (RCT): the FRUIT-RCT. We investigated whether low-molecular-weight heparin (LMWH), when added to low-dose aspirin, influenced fetal growth and flow-velocity in uterine and umbilical arteries. The FRUIT-RCT included 139 women with a previous delivery before 34 weeks gestation with HD and/or a small-for-gestational-age (SGA) infant and inheritable thrombophilia. Ultrasound measurements were performed at 22-24, 28-30 and 34-36 weeks gestation. This specific population has an impressively high risk both for neonatal SGA (30%) and for decreased flow-velocity within the uterine artery (48%). We concluded that the addition of LMWH to aspirin did not influence either fetal growth and umbilical artery flow-velocity over time nor uterine artery flow-velocity.

In **Chapter 2** we investigated in a cohort study disease activity around and during pregnancy and pregnancy outcome in women with systemic lupus erythematosus (SLE) taking their antiphospholipid antibody status into account. Moreover, differences between first and consecutive pregnancies and number of live births were examined. We included all ongoing pregnancies (>16 weeks gestation) of SLE patients receiving joint care from rheumatologists and gynecologists in two tertiary centers in the Netherlands between 2000-2015. From 96 women (84% Caucasian), 144 pregnancies were included. The median SELENA-SLE(P)DAI score was 2 before (<6 months), during and after pregnancy (<6 months) and flare rates were 6.3%, 20.1% and 15.3% respectively. HD, intrauterine fetal death (IUFD), preterm birth and SGA infants occurred in 18.1%, 4.1%, 32.7% and 14.8% respectively. Only HELLP-syndrome occurred more often in women with SLE and antiphospholipid syndrome (APS) compared to SLE women with or without antiphospholipid antibodies. Pregnancy complication rates were similar in first and consecutive pregnancies and half of the women did not experience any pregnancy complication during their studied reproductive period, whereas 42.7% developed a complication during all pregnancies. Mean number of pregnancies was 2.4 and live births 1.7. In conclusion, in a multidisciplinary monitored SLE population with low disease

activity, maternal and perinatal complications were nearly equally distributed between women with SLE with or without antiphospholipid antibodies or APS. This was irrespective of antiphospholipid antibody status and irrespective of first and consecutive pregnancies. We should use this information for patient counseling.

In **Chapter 3** we related the use of aspirin and/or LMWH to pregnancy complications in women with SLE and primary APS. We studied 184 ongoing pregnancies, in which women had their check-ups on both the obstetric and rheumatology department in one of two Dutch tertiary centres between 2000-2015. LMWH and aspirin was prescribed in 15/109 SLE women without antiphospholipid antibodies (aPL), 5/14 with aPL, 11/13 with APS, 45/48 with primary APS. Main complications in the four treatment groups (no anticoagulant treatment, aspirin, LMWH, aspirin and LMWH) included HD (9.4%, 23.3%, 50%, 18.4% respectively) and preterm birth (16.7%, 34.3%, 75%, 36.8% respectively). The maternal and perinatal outcomes in the complete cohort showed that the subgroups with anticoagulant treatment experienced more maternal and perinatal complications compared to those without anticoagulant therapy. The overall incidence of maternal and perinatal complications was high, irrespective of treatment group and despite low SLE disease activity in the majority of the population within six months before pregnancy.

In **Chapter 4** we describe the protocol of an individual patient data meta-analysis (IPDMA) for LMWH intervention for the prevention of recurrent placenta-mediated pregnancy complications. Placenta-mediated pregnancy complications include HD, late pregnancy loss, placental abruption, and SGA infants. We conducted a systematic review to identify RCT's with LMWH intervention for the prevention of recurrent placenta-mediated pregnancy complications. Investigators and statisticians representing eight trials met to discuss the outcomes and analysis plan for an IPDMA. The goal of the IPDMA is a thorough estimation of treatment effects in patients with prior individual placenta-mediated pregnancy complications and exploration of which complications are specifically prevented by LMWH.

In **Chapter 5** the results of the IPDMA are presented. A systematic review was performed in May, 2013, which identified eight eligible randomised trials done between 2000 and 2013 of LMWH to prevent recurrent placenta-mediated pregnancy complications. We analysed data from 963 eligible women in eight trials: 480 randomly assigned to LMWH and 483 randomly assigned to no LMWH. Participants were mostly white (88%) with a mean age of 30.9 years and 42% had thrombophilia. The inclusion criteria were preeclampsia, placental abruption, SGA infant, pregnancy loss above 16 weeks or two pregnancy losses above 12 weeks. The primary outcome was preeclampsia below 34 weeks, severe preeclampsia, SGA infant, pregnancy loss above 20 weeks or placental abruption. In the primary analysis, LMWH did not significantly reduce the risk of recurrent placenta-mediated pregnancy complications (LMWH 14% versus no LMWH 22%). We noted significant heterogeneity between single-centre and multicentre trials. In subgroup analyses, LMWH in multicentre trials reduced the primary outcome in women with previous abruption but not in any of the other subgroups

of previous complications. We concluded that LMWH does not seem to reduce the risk of recurrent placenta-mediated pregnancy complications in at-risk women.
In **Chapter 6**, we examined adherence rates in women to whom aspirin is prescribed due to their increased risk for pregnancy complications. Women between 24 and 36 weeks gestation with an indication for aspirin use during pregnancy were invited for this study. A survey was used which included two validated questionnaires, the simplified medication adherence questionnaire (SMAQ) and the Beliefs and Behaviour Questionnaire (BBQ). Indications for aspirin use during pregnancy were previous HD, fetal growth restriction, IUFD or current maternal disease. Non-adherence rates according to the SMAQ and BBQ were 46.3% and 21.4% respectively. No differences in demographic background or obstetrical characteristics between adherent and non-adherent women could be demonstrated. This study showed that adherence for aspirin in this high-risk population cannot be taken for granted. Surprisingly, despite the clear short term to prevent recurrent pregnancy complications, the non-adherence rates in pregnant women are comparable with the non-adherence rates for aspirin in the non-pregnant population. In daily practice, the doctor should motivate their patients to take their aspirin.

In **Chapter 7** we examined whether aspirin resistance is associated with recurrent HD. We hypothesized that the aspirin did not work properly in women with recurrent HD. Aspirin resistance was tested using three complementary tests: PFA-200, VerifyNow® and serum thromboxane B_2 (TXB_2). Thirteen of 24 women with recurrent HD and 16 of 24 women without recurrent HD participated. The prevalence of aspirin resistance in the whole group was 34.5% according to the PFA-200, 3.4% according to the VerifyNow® and 24.1% according to TXB_2. The prevalence of aspirin resistance by any test was 51.7%. Aspirin resistance per individual test did not differ between women with and without recurrent HD. Aspirin resistance measured by any test occurred more frequently in women without recurrent HD irrespective of LMWH.A limitations of the study was that it was performed up to 16 years after the pregnancy. In conclusion, we could not find a relation between recurrent HD and aspirin resistance per test, measured up to 16 years after pregnancy. On the contrary, complementary aspirin resistance measurements were encountered more frequently in women without recurrent HD.

In **Chapter 8** we present the protocol of the RADAR study: Resistancy of Aspirin During and After pRegnancy. This longitudinal cohort study is undertaken to investigate the above mentioned limitation and assess the consistency of aspirin resistance during and after pregnancy. It is unknown whether aspirin resistance changes over time and how pregnancy affects aspirin resistance. Aspirin resistance is measured in the first, second and third trimester of pregnancy and at least three months postpartum. Four complementary tests are used: PFA-200, VerifyNow®, Chrono-log light transmission aggregometry and serum thromboxane B_2. 23 women participated in this study. The results are expected during the first half of 2017. The results will support us with the interpretation of the results of Chapter 7 on the possible relation between aspirin resistance and HD. Adding the knowledge of the

present study with the use of several complementary tests facilitates comparison with other studies and thus might give more insight if aspirin resistance is a factor of importance in the treatment to prevent HD.

In **Chapter 9** the prevalence of cardiovascular risk factors were examined in women with inheritable thrombophilia 8-19 years after early-onset HD, with or without recurrent HD. We included 22 women: 11 women with recurrent HD and the other 11 women without recurrent HD. Nearly three-quarters of the women had an higher prevalence of cardiovascular risk factors, irrespective of single or recurrent HD. Women with recurrent HD did have higher systolic and diastolic blood pressure and albumin creatinine ratio compared to women with single HD. This is similar to other studies examining cardiovascular risk factors in women with a history of HD in which their thrombophilia status was unknown.

In **Chapter 10** we hypothesized that women who have had a pregnancy complicated by HD are at increased risk of Alzheimer's Disease later in life. We performed a nested cohort study in 251 women with Alzheimer's Disease and 249 women without Alzheimer's Disease. Neither a significant difference between women with and without Alzheimer's Disease in a history of HD, including pregnancy induced hypertension (12,7 vs 25.9% respectively) was found. Nor an association between Alzheimer's Disease and preeclampsia (2.9% in women with Alzheimer's Disease versus 3.1% in women without Alzheimer's Disease). We concluded that a reported history of HD appears not to be associated with Alzheimer's Disease later in life. These findings suggest (at least partly) different pathophysiological pathways of cerebrovascular damage associated with HD and those related to Alzheimer's Disease.

In the **discussion**, the results of this thesis are discussed and recommendations for clinical practice and implications for future research are debated and summarized hereafter.
First, the IPDMA, only found a beneficial effect of LMWH in women with previous placental abruption, which is not in line with the results of individual RCT's which are included in this IPDMA. Limitation of the IPDMA is the use of composite in- and exclusion criteria. Second, to examine the effect of LMWH in women with SLE, a RCT is needed. Third, adherence for aspirin is a subject for patient counselling. Moreover, the hypothesis that aspirin resistance is involved in the occurrence or recurrence of HD seems plausible, it might be possible that the right device/test to examine aspirin resistance has not been developed yet. Furthermore, to examine the role of thrombophilia itself on the risk to develop cardiovascular risk factors after HD can be examined in the near future, since data of a similar population without thrombophilia is currently collected. Finally, to examine if there is a relation between Alzheimer's Disease and HD, and to eliminate the potential recall bias, a prospective cohort study is needed.

Nederlandse Samenvatting

Nederlandse samenvatting

In dit proefschrift wordt het gebruik van aspirine (een bloedverdunner in tabletvorm) en laag-moleculair-gewicht heparine (een injectie die zorgt voor antistolling van het bloed) in de zwangerschap onderzocht om onder andere zwangerschapsvergiftiging (een ziekte die gerelateerd is aan problemen met de placenta) te voorkomen. De populatie die onderzocht is had een verhoogd risico op een zwangerschapsvergiftiging doordat zij dit eerder hebben meegemaakt, of doordat zij een onderliggende aandoening hebben zoals systemische lupus erythematosus. Een zwangerschapsvergiftiging kan zich uiten als een pre-eclampsie (hoge bloeddruk met eiwit verlies in de urine), eclampsie (stuipen, wat lijkt op een epileptische aanval) of HELLP syndroom (waarbij er onder andere lage bloedplaatjes zijn en de lever niet goed functioneert). Daarnaast worden risicofactoren voor hart- en vaatziekten na zwangerschapsvergiftiging onderzocht in een specifieke groep: vrouwen met trombofilie (een stollingsstoornis waarbij het bloed te snel stolt). Tevens wordt er onderzocht of er een relatie is tussen zwangerschapsvergiftiging en de ziekte van Alzheimer.

In de **introductie** worden risicofactoren, preventieve maatregelen en korte en lange termijn uitkomsten van zwangerschapsvergiftiging besproken, alsmede de doelen van dit proefschrift. In **hoofdstuk 1** hebben we onderzocht of laag-moleculair-gewicht heparine, indien toegevoegd aan aspirine, de groei van de foetus en de doorstroming van de bloedvaten van de baarmoeder en navelstreng beïnvloedt. Dit werd onderzocht in de FRUIT-RCT hebben 139 vrouwen deelgenomen die eerder een bevalling vóór 34 weken hadden vanwege of een zwangerschapsvergiftiging of bevallen zijn van een klein kind (<p10). Bovendien hadden ze trombofilie. Tijdens dit onderzoek werd op drie momenten een echo gemaakt: tussen 22-24 weken, 28-30 weken en 34-36 weken. De conclusie van dit onderzoek is dat de toevoeging van laag-moleculair-gewicht heparine bij aspirine noch invloed heeft op de groei van de foetus noch op de doorstroming van de baarmoeder- en navelstrengbloedvaten. In deze gehele populatie was er een hoog risico op het krijgen van een te klein kind (30%) en op een verminderde doorstroming van het bloedvat naar de baarmoeder (48%).

In **hoofdstuk 2** presenteren we de uitkomsten van een onderzoek waarbij we zwangerschappen van vrouwen met de ziekte SLE (systemische lupus erythematosus, een auto-immuunziekte) hebben onderzocht. We keken naar zwangerschapsuitkomsten en ziekteactiviteit voor, tijdens en na de zwangerschap. Hierbij hebben we rekening gehouden met de antifosfolipiden status (ook een mate van stollingsstoornis). Verder hebben we naar verschillen in het complicatierisico tussen een eerste en tweede zwangerschap gekeken. Tevens hebben we gekeken hoeveel levende kinderen deze vrouwen hebben. Voor dit onderzoek hebben vrouwen deelgenomen die langer dan 16 weken zwanger zijn geweest tussen 2000 en 2015 en onder controle waren in het VUmc Amsterdam of UMC Utrecht bij zowel de reumatoloog als de gynaecoloog. In totaal hebben 96 vrouwen (84% Kaukasisch) met 144 zwangerschappen deelgenomen. De SLE ziekteactiviteit was laag vóór, tijdens en na de zwangerschap. De kans op opleving van de SLE ziekte was voor de zwangerschap 6,3%, tijdens de zwangerschap 20,1% en na de

zwangerschap 15,3%. In de hele populatie kreeg 18,1% zwangerschapsvergiftiging, had 4,1% een overleden foetus, 32,7% een vroeggeboorte en 14,8% een klein kind. Het HELLP syndroom (een ernstige vorm van zwangerschapsvergiftiging) trad vaker op bij vrouwen met SLE die ook het antifosfolipiden syndroom (stollingsstoornis) hadden in vergelijking met vrouwen met SLE die dat niet hadden. Het risico op een zwangerschapscomplicatie bleef gelijk tussen eerste en latere zwangerschappen. Dit wijkt af van de normale populatie, waarbij dit risico afneemt in latere zwangerschappen. De helft van alle vrouwen met SLE had nooit een complicatie in een van al haar zwangerschappen, tegenover 42,7% die een complicatie kreeg in alle zwangerschappen. Het gemiddeld aantal zwangerschappen was 2,4 en er waren gemiddeld 1,7 levende kinderen per vrouw. We concludeerden dat de zwangerschapscomplicaties onafhankelijk waren van de antifosfolipiden status en onafhankelijk was van een eerste of volgende zwangerschap. Deze informatie kunnen we gebruiken in de voorlichting van patiënten.

In **hoofdstuk 3** hebben we het gebruik van aspirine en laag-moleculair-gewicht heparine gerelateerd aan zwangerschapscomplicaties in vrouwen met SLE en/of het antifosfolipiden syndroom. We onderzochten 184 zwangerschappen waarbij de vrouwen tussen 2000 en 2015 onder controle waren in het VUmc Amsterdam of UMC Utrecht bij zowel de reumatoloog als de gynaecoloog. Laag-moleculair-gewicht heparine met aspirine is voorgeschreven aan 15/109 vrouwen met SLE zonder antifosfolipiden antilichamen in het bloed, aan 5/14 met SLE en antifosfolipiden antilichamen, aan 11/13 met SLE en antifosfolipiden syndroom en aan 45/48 met primair antifosfolipiden syndroom zonder SLE. We hebben alle vrouwen opgedeeld in 4 behandel groepen: geen behandeling met aspirine of laag-moleculair-gewicht heparine, behandeling met alleen aspirine, behandeling met alleen laag-moleculair-gewicht heparine en behandeling met zowel aspirine als laag-moleculair-gewicht heparine. Belangrijkste complicaties waren zwangerschapsvergiftiging (9,4%, 23,3%, 50%, 18,4% in groep 1-4) en vroeggeboorte (16,7%, 34,3%, 75%, 36,8% in groep 1-4). Vrouwen die behandeld waren met aspirine en/of laag-moleculair-gewicht heparine hadden meer complicaties dan vrouwen zonder deze medicijnen. Ongeacht in welke behandelgroep vrouwen zaten, waren er veel complicaties in de gehele groep bij zowel moeder als kind.

In **hoofdstuk 4** beschrijven we het protocol van een onderzoek waarbij de uitkomsten van meerdere gerandomiseerde onderzoeken worden gebundeld. Het onderwerp van deze onderzoeken was het effect van laag-moleculair-gewicht heparine in de zwangerschap. Hoofdonderzoekers en statistici van acht gerandomiseerde trials hebben de primaire, secundaire en overige uitkomsten en analyses besproken. Het doel van dit onderzoek (een IPDMA: individualized patient data meta-analysis) is om te kijken welke vrouwen met eerdere placenta gerelateerde zwangerschapscomplicaties baat hebben bij laag-moleculair-gewicht heparine. De uitkomsten van dit onderzoek worden gepresenteerd in hoofdstuk 5.

In **hoofdstuk 5** zijn de resultaten van deze IPDMA gepresenteerd. Er werden acht geschikte gerandomiseerde onderzoeken tussen 2000 en 2013 gevonden (door middel van een literatuuronderzoek), die het effect van laag-moleculair-gewicht heparine op placenta

gerelateerde zwangerschapscomplicaties hebben onderzocht. Data van 963 vrouwen uit acht trials zijn geanalyseerd: 480 vrouwen kregen laag-moleculair-gewicht heparine en 483 vrouwen niet. De meeste vrouwen waren Kaukasisch (88%) met een gemiddelde leeftijd van 30,9 jaar. Een vorm van trombofilie was aangetoond in 42% van de vrouwen. De vrouwen hadden een ernstige complicatie in een eerdere zwangerschap, bijvoorbeeld een zwangerschapsvergiftiging, loslating van de placenta of een overleden foetus. De primaire uitkomst was een samenstelling van meerdere complicaties: zwangerschapsvergiftiging, een te klein kind, een overleden foetus en loslating van de placenta. Al deze complicaties worden in verband gebracht met een niet goed functionerende placenta. Uit de primaire uitkomst bleek dat laag-moleculair-gewicht heparine niet het risico op placenta gerelateerde zwangerschapscomplicaties verminderde . De laag-moleculair-gewicht heparine groep had 14% risico op placenta gerelateerde zwangerschapscomplicaties, de groep zonder laag-moleculair-gewicht heparine 22% risico. Het verschil tussen beide groepen was niet significant). Er werd een opvallend verschil tussen single-center en multicenter trials gevonden. In subgroep analyse vonden we in de multicenter trials dat alleen vrouwen met een placenta loslating in de voorgeschiedenis baat hadden bij laag-moleculair-gewicht heparine. De algemene conclusie was dat laag-moleculair-gewicht heparine niet het risico verkleint op placenta gerelateerde zwangerschapscomplicaties.

In **hoofdstuk 6** hebben we de therapietrouw van aspirine in de zwangerschap onderzocht bij vrouwen met een hoog-risico zwangerschap. Vrouwen die aspirine in de zwangerschap kregen, werden voor dit onderzoek uitgenodigd tussen 24 en 36 weken zwangerschapsduur. Er is een vragenlijst opgesteld die twee gevalideerde vragenlijsten bevatte: de *simplified medication adherence questionnaire* (SMAQ) en de *Beliefs and Behaviour Questionnaire* (BBQ). Indicaties voor het gebruik van aspirine in de zwangerschap waren een eerdere zwangerschapsvergiftiging, eerdere groeivertraging of dode foetus is een eerdere zwangerschap. Ook een ziekte van de moeder kon een reden voor aspirine gebruik zijn. Therapieontrouw was 46,3% volgens de SMAQ en 21.4% volgens de BBQ. Er waren geen verschillen in kenmerken tussen vrouwen die wel en niet therapietrouw waren. Dit onderzoek laat zien dat therapietrouw voor aspirine in de zwangerschap niet zomaar aangenomen moet worden. Deze percentages komen overeen met therapietrouw voor aspirine in niet-zwangere populaties.

In **hoofdstuk 7** presenteren we de uitkomsten van ons onderzoek naar een relatie tussen aspirineresistentie en het ontstaan van zwangerschapsvergiftiging. Aspirine wordt in de zwangerschap onder andere voorgeschreven aan vrouwen die eerder een zwangerschapsvergiftiging hebben doorgemaakt. Bij sommige vrouwen treedt opnieuw zwangerschapsvergiftiging op, ondanks dat zij aspirine gebruiken om dit risico te verkleinen. Onze hypothese was dat aspirine niet goed werkt bij vrouwen die opnieuw een zwangerschapsvergiftiging doormaakten en dat zij aspirine resistent zijn. Wij hebben hiervoor drie testen gebruikt die aspirineresistentie meten (de PFA-200, de VerifyNow en door tromboxaan B_2 in het serum te meten). Dertien vrouwen hadden een zwangerschapsvergiftiging gehad tijdens behandeling met aspirine en zestien vrouwen hadden geen zwangerschapsvergiftiging gehad. In de hele groep was

34,5% aspirineresistent volgens de PFA-200, 3,4% volgens de VerifyNow en 24,1% volgens het tromboxaan B_2. In totaal was 51,7% resistent voor aspirine als we als definitie hanteerden dat ze aspirineresistent waren volgens één van de drie testen. Aspirineresistentie per test apart verschilde niet tussen vrouwen met en zonder zwangerschapsvergiftiging. Als alle testen samen werden genomen kwam aspirineresistentie vaker voor bij vrouwen zonder zwangerschapsvergiftiging. Een beperking van dit onderzoek is dat het onderzocht werd tot 16 jaar na de zwangerschap en niet tijdens de zwangerschap. Concluderend konden we geen relatie vinden tussen aspirineresistentie en zwangerschapsvergiftiging per test apart gemeten. Aan de andere kant trad aspirineresistentie vaker op in vrouwen zonder zwangerschapsvergiftiging wanneer alle testen samen genomen werden.

In **hoofdstuk 8** presenteren we het protocol van de RADAR study: *Resistancy of Aspirin During and After pRegnancy*, in het Nederlands vertaald aspirineresistentie tijdens en na de zwangerschap. Dit onderzoek is nog gaande en wordt uitgevoerd om te kijken of aspirineresistentie consistent optreedt tijdens en na de zwangerschap. Dit onderzoek is opgezet om de genoemde beperking in hoofdstuk 7 te onderzoeken. Verandert aspirineresistentie tijdens en na de zwangerschap? Hoe vaak komt aspirineresistentie voor? Hiertoe wordt aspirineresistentie gemeten in het eerste, tweede en derde trimester van de zwangerschap en meer dan drie maanden na de zwangerschap. We gebruiken vier testen die elkaar aanvullen: de PFA-200, VerifyNow, Chrono-log LTA en we meten het tromboxaan B_2 niveau in serum. 23 vrouwen hebben deelgenomen aan dit onderzoek. De resultaten worden in de eerste helft van 2017 verwacht. Deze resultaten zullen ons helpen de uitkomsten van hoofdstuk 7 beter te begrijpen: is er een relatie tussen aspirineresistentie en zwangerschapsvergiftiging. Daarnaast zullen de resultaten van dit onderzoek het mogelijk maken de uitkomsten te vergelijken met andere onderzoeken. Dit doordat we meerdere testen naast elkaar gebruiken. Zo hopen we meer te weten te komen of aspirineresistentie een factor is in de behandeling van zwangerschapsvergiftiging.

In **hoofdstuk 9** worden risicofactoren op hart- en vaatziekten in kaart gebracht bij vrouwen met een erfelijke vorm van trombofilie die 8 tot 19 jaar eerder een zwangerschapsvergiftiging hebben gehad die voor 34 weken zwangerschapsduur ontstond. In totaal hebben 22 vrouwen deelgenomen: elf vrouwen hebben na de eerste zwangerschapsvergiftiging nog een zwangerschap gehad die gecompliceerd werd door een zwangerschapsvergiftiging. Elf vrouwen hadden in een vervolg zwangerschap niet opnieuw een zwangerschapsvergiftiging. Ongeveer driekwart van de vrouwen had een verhoogde prevalentie van risicofactoren voor hart- en vaatziekten, ongeacht of zij één keer of twee keer zwangerschapsvergiftiging hebben gehad. Vrouwen die twee keer zwangerschapsvergiftiging hebben doorgemaakt, hadden een hogere boven- en onder bloeddruk en een hogere albumine creatinine ratio (dit zegt iets over de nierfunctie) vergeleken met vrouwen die één keer een zwangerschapsvergiftiging hebben doorgemaakt. Deze uitkomsten zijn vergelijkbaar met onderzoeken waarbij risicofactoren op hart- en vaatziekten na zwangerschapsvergiftiging zijn onderzocht bij vrouwen zonder trombofilie.

In **hoofdstuk 10** was onze hypothese dat een zwangerschap gecompliceerd door zwangerschapsvergiftiging een verhoogd risico geeft op het ontwikkelen van Alzheimer op oudere leeftijd. Er hebben 251 vrouwen met Alzheimer en 249 vrouwen zonder Alzheimer mee gedaan. We vonden geen verschil in zwangerschapsvergiftiging in de voorgeschiedenis tussen vrouwen met en zonder Alzheimer (2.9% met Alzheimer had een zwangerschapsvergiftiging gehad versus 3.1% zonder Alzheimer). We concludeerden dat zwangerschapsvergiftiging, gerapporteerd door de patiënt zelf, niet geassocieerd lijkt met Alzheimer. Deze bevinding suggereert (in ieder geval deels) een verschillend ontstaan van zwangerschapsvergiftiging en Alzheimer.

In de **discussie** wordt gereflecteerd op de uitkomsten van dit proefschrift en wordt er gekeken naar de betekenis van de resultaten voor de klinische praktijk en worden ideeën voor verder onderzoek genoemd.

Curriculum Vitae

Curriculum Vitae

Written by Anouk Bokslag and Irma Scholtens

Carolien Abheiden was born on 2 June 1986 in Zwolle. She grew up with her parents and two younger sisters in Glimmen, in the northern province of Groningen. Carolien turned out to be an energetic girl, with a love for equestrian sports, which she practised at a national level.
After receiving her high school diploma (VWO, highest level of high school education) at the Maartens College in Haren, she went on to study medicine at the University of Groningen (RUG). Carolien, an avid and natural student, chose to really immerse herself in her studies and extracurricular programs. Examples of this are her junior doctor internship in obstetrics in Surabaya, Indonesia and completing the optional project 'Organisation & Management in a Hospital'. In addition, she developed a passion for rowing at the rowing club Gyas, where she also became a member of the board. In 2012, she graduated cum laude at the RUG.
After completing her junior doctor internship in obstetrics and gynaecology and her scientific internship with Prof. dr. Hanneke de Vries in Amsterdam, Carolien felt right at home in the VU University Medical Center Amsterdam (VUmc). Her professional career started when she became an ANIOS (MD) in the IJsselland hospital in Capelle aan den IJssel. After a year, Carolien continued her residency in the VUmc, where she was also able to continue - initially part-time and later full-time - with the research that she started during her scientific internship. Under the direction of Prof. dr. Hanneke de Vries, Dr. Marjon de Boer and Dr. Abel Thijs, the research was rapidly expanded to an official PhD program, the result of which now lies before you.
When Carolien's husband was offered to work on a project in Kenya, she decided to accompany him and spend half a year of her PhD program in Nairobi, Kenya. She continued her PhD research in Kenia and wrote a business plan for a child delivery centre outside of Nairobi. She also visited several parent/child-projects for a Dutch non-profit organization. In April 2016, she started her training as a gynaecologist in the OLVG west (with dr. Janet Kwee) within the cluster VUmc (with Prof. dr. Hanneke de Vries).
Carolien is married to Henk Veldman and they have a daughter, Mia, who was born in the summer of 2016.
Considering all of Carolien's extraordinary qualities, we trust that you will hear more about our amazing, hard-working and smart friend in the future.

Curriculum Vitae

Geschreven door Anouk Bokslag en Irma Scholtens

Carolien Abheiden werd op 2 juni 1986 geboren in Zwolle als eerste kind van het gezin. Zij groeide met haar ouders en twee zusjes op in het Groningse Glimmen. Carolien was toen al erg actief met een liefde voor paardensport, die zij op nationaal niveau beoefende.
Nadat zij haar VWO-diploma behaalde aan het Maartens College in Haren, ging Carolien Geneeskunde studeren aan de Rijksuniversiteit Groningen (RUG). Deze studie doorliep zij met plezier en gemak, waarbij ze koos voor zowel verdieping ín de studie als naast de studie. Zo liep zij een semi-arts stage verloskunde in Surabaya, Indonesië en volbracht zij een keuzeproject Organiseren & Managen in een ziekenhuis. Daarnaast was zij ook veel te vinden op roeivereniging Gyas, waar ze onder meer bestuurslid was. In 2012 studeerde zij cum laude af aan de RUG.
Na het afronden van haar semi-arts stage verloskunde en gynaecologie en de wetenschappelijke stage bij Prof. dr. Hanneke de Vries in Amsterdam voelde Carolien zich helemaal thuis in het VU medisch centrum (VUmc). Haar professionele carrière startte als ANIOS in het IJsselland Ziekenhuis in Capelle aan den IJssel. Na een jaar zette Carolien haar ANIOS-schap voort in het VUmc en kon zij, eerst deeltijds, later voltijds, verder met het onderzoek waar zij in haar wetenschappelijke stage mee was begonnen. Onder leiding van Prof. dr. Hanneke de Vries, dr. Marjon de Boer en dr. Abel Thijs werd het onderzoek al snel uitgebreid naar een officieel promotietraject, waarvan het resultaat voor u ligt.
Toen de man van Carolien via zijn werk een project aangeboden kreeg in Kenia, besloot zij mee te reizen en een half jaar van het promotietraject door te brengen in Nairobi, Kenia. Hier werkte zij verder aan haar promotieonderzoek en schreef daarnaast ook een businessplan voor een bevalcentrum buiten Nairobi. Ook bezocht zij diverse moeder/kind-projecten voor een Nederlandse non-profit organisatie. Zij startte in april 2016 met haar opleiding tot gynaecoloog in het OLVG west (opleider dr. Janet Kwee) binnen het cluster VUmc (opleider Prof. dr. Hanneke de Vries).
Carolien is getrouwd met Henk Veldman en in de zomer van 2016 werd hun dochter, Mia, geboren.
Gezien alle uitzonderlijke kwaliteiten van Carolien vertrouwen wij erop dat u in de toekomst nog veel van onze sprankelende, ondernemende en slimme vriendin zult horen.

Dankwoord

Dankwoord

Dit proefschrift kon niet ontstaan zonder hulp van anderen. Graag wil ik een aantal mensen bedanken.
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- **Prof. dr. J.I.P. de Vries**, beste Hanneke, wat heb ik ontzettend veel van jou geleerd. In 2011 kwam ik als student mijn stage wetenschap bij jou doen, achteraf gezien het begin van mijn proefschrift. Na liters rooibos thee, veel Skype afspraken terwijl ik in Nairobi zat en een mooie trip naar Canada is dit het resultaat. Zonder jouw inzet was dit zeker niet gelukt. Je bent een ontzettend lieve en betrokken promotor, wat de samenwerking heel plezierig maakt. Ik hoop dat we in de toekomst net zo blijven samenwerken, ook in onze nieuwe rollen als opleider en AIOS.
- **dr. M.A. de Boer**, beste Marjon, in 2014 werd jij officieel mijn co-promotor en dat bleek een schot in de roos. Je bent ontzettend enthousiast en kwam snel met nieuwe ideeën voor mijn proefschrift. Verder heb je altijd een blik op het einddoel gehouden en mij hierin geadviseerd. De plezierige samenwerking kwam ook door je vrolijkheid, betrokkenheid en altijd snelle reacties. Heel erg bedankt voor alles.
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