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Study design

HABIT: Heparin and Aspirin on Birth in Inherited Thrombophilia, an International Multicenter Phase IIb Randomized Triple Blinded Clinical Trial

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ABSTRACT:

Introduction: In pregnant women with inherited thrombophilia (IT) and recurrent pregnancy loss, there is no higher-level evidence proving the beneficial effects of anticoagulation or platelet inhibition in preventing miscarriages. We hypothesize that anticoagulation with low molecular weight heparin (LMWH) and/or platelet aggregation inhibition with aspirin will increase the proportion of live birth in this population.

Methods: In this phase IIb, factorial, randomized, triple blinded, placebo-controlled (double dummy) clinical trial, pregnant women aged 18 to 40 with a history of IT and 2 or more previous miscarriages, will be randomized and stratified by age and number of miscarriages in a 2x2 factorial design will be allocated equally to one of the four arms. The primary outcome of live birth will be analyzed through logistic regression analysis, controlling for strata, and results will be reported as odds ratio (OR) and 95% confidence intervals (CIs). Similarly, the secondary outcomes will include pregnancy loss, maternal mortality, major bleeding events, medication-associated adverse events, placental abruption, preterm birth, and gestational age at delivery. We will perform subgroup analysis for smoking status, weight, age, number of miscarriages, and type of thrombophilia.

Discussion: There is lack of evidence for the use of anticoagulants to prevent pregnancy loss in women with inherited thrombophilia, despite the common diverging prescribing practice predominantly extrapolated from observations in acquired thrombophilia. We aim to provide an evidence base to create a standard of care in cases of recurrent pregnancy loss in women with IT.

Keywords: heparin; aspirin; inherited thrombophilia; pregnant; live birth.

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Introduction

Inherited thrombophilia (IT), a clinical condition characterized by congenital predisposition to the development of multiple thromboembolic episodes of venous thromboembolism (Franchini, 2006). It includes factor V Leiden and prothrombin gene mutation, deficiency of protein C and protein S, deficiency of antithrombin III, hyperhomocysteinemia, and methylenetetrahydrofolate reductase mutations (James, 2005). IT may be associated with negative events during pregnancy (Wu, 2006), as recurrent pregnancy loss (Pabinger, 2005; Pabinger, 2009; Middeldorp, 2007). The American Society for Reproductive Medicine defines recurrent pregnancy loss as two or more clinical pregnancy losses, not necessarily consecutive (Middeldorp, 2007). This condition affects 2%–5% of pregnant women (Practice Committee of the American Society for Reproductive Medicine, 2012) and, in pregnant women with inherited thrombophilia, the risk of miscarriage is estimated to be 1.5-2 times higher than in the overall population, which amounts an overall risk of miscarriage of 40% (de Jong, 2014; Dimitriadis, 2020; Ford, 2009).

Although thrombophilia is associated with recurrent pregnancy loss and to other aversive obstetric outcomes, including preeclampsia, intrauterine growth restriction and stillbirths, the pathophysiological causality is still not fully understood (Pabinger, 2005; Pabinger, 2009; Middeldorp, 2007; Visser, 2011; Abu-Heija, 2014). It is postulated that pregnancy itself causes a hypercoagulable state and that the additional imbalance towards hypercoagulation caused by thrombophilia may impair blood flow through the maternal veins. This leads to clots in the placental blood vessels and ultimately to fetal growth restriction and/or fetal demise (Colman-Brochu, 2004).

Studies have shown that anticoagulants, as low molecular weight heparin (LMWH) and aspirin, are potential interventions for women with recurrent miscarriages and thrombophilia. LMWH is often used during pregnancy with a good safety profile. The mechanisms of action include an anti-inflammatory effect and modulation of angiogenic pathways (Brenner, 2007). Aspirin inhibits the synthesis of prostaglandins, and 75–100 mg/day are sufficient to revert acetylates serine 530 of cyclooxygenase 1, inhibiting platelet generation of thromboxane-A2, resulting in an antithrombotic effect.

Currently, prophylactic anticoagulation is mostly evaluated on a case-by-case basis, if inherited or acquired thrombophilias are present, and depending on the history of prior pregnancies and their outcomes (Battinelli, 2013). A recent systematic review showed that the combination of heparin (unfractionated heparin or LMWH) plus aspirin may increase the proportion of live birth in women with persistent antiphospholipid antibody syndrome, compared with aspirin treatment alone (Hamulyák, 2020). Another systematic review concluded that there is limited evidence on efficacy and safety of aspirin and heparin in women with recurrent pregnancy losses with or without inherited thrombophilia, but the studies were not powered enough to assess the specific population with inherited thrombophilia (de Jong, 2014).

Thus, using anticoagulants for women with a history of recurrent miscarriages remains controversial, despite the commonality of screening for diseases linked to thromboembolism status and prescription of anticoagulants treatments among clinicians (Gee, 2014; Signorelli, 2019). While the safety of aspirin and heparin has been demonstrated in pregnancy (de Jong, 2014; Hamulyák, 2020), the efficacy of anticoagulants in women with recurrent miscarriages and inherited thrombophilia needs to further be studied through larger randomized controlled trials (de Jong, 2014; Battinelli, 2013).There are no past trials addressing this issue for this specific population, and the only ongoing trial compares heparin to no treatment (de Jong, 2015). In contrast, our proposal is to evaluate the effects of aspirin and heparin alone and in combination on live birth, in comparison to placebo.

This is a triple blinded placebo controlled phase IIb clinical trial investigating the proportion of live birth among pregnant women with recurrent miscarriages and IT treated with enoxaparin (a LMWH) and aspirin for the duration of the pregnancy, compared to those treated with each drug alone and placebo. We hypothesize that combining enoxaparin and aspirin, or each intervention alone, will be associated with a higher proportion of live birth, as compared with the placebo group.

Materials and Methods

Trial Design

This is a phase IIb, factorial, randomized, triple blinded, placebo-controlled (double dummy) clinical trial. The subjects will be randomized in a 2x2 factorial

design to one of the following arms, in an equal allocation ratio: LMWH 40mg subcutaneously (SC) once daily plus aspirin 100 mg orally once daily; LMWH 40mg SC once daily plus placebo aspirin orally once daily; placebo LMWH SC once daily plus aspirin 100 mg orally once daily; or placebo LMWH SC once daily plus placebo aspirin orally once daily. It will begin after the approval by Institutional Review Boards and Ethics Committees of the institutions involved, as well as the informed consent of the women and their partners, if required.

Study Setting

It will be an international multicenter clinical trial, aiming at patient recruitment from specialized centers in infertility treatment, women's/maternity hospitals and university/academic hospitals in North America, Central and South America, Europe and Asia. To enhance recruitment and stratify the participants by demography, we aim to have 20 participating sites evenly distributed based on patient availability at clinical centers.

Randomization

The study will be conducted using 2x2 factorial design and subjects will be randomized by stratified permuted blocks in multiples of 4, with a maximum block size of 12. Stratification will be by age (≥36 years and <36 years), previous number of miscarriages (two or more than two) and by location. The Duke Clinical Research Institute (DCRI) will serve as a data coordinating center and provide allocation by site request, via web interface. The random allocation will be concealed for the patient and the treating physician meanwhile randomization code will be accessed only by a secure password which will be known by an independent staff member.

The participants will be enrolled by the treating physician, after confirmation of eligibility criteria and signing informed consent. An independent research staff will assign the intervention according to the obtained allocation sequence. The DCRI will serve as a central database to randomize subjects and store subject data across all participating sites.

Blinding

This will be a triple blinded study, since participants, treating physicians, and statisticians will not be aware of group allocation. This will be accomplished by a double dummy design, including placebo aspirin pills and placebo enoxaparin injections, indistinguishable from the active treatments. Both active and placebo drugs will be provided by the pharmaceutical company, but identically labeled and packaged by clinical trial pharmacy personnel of participant sites, which will remain unblinded.

The following conditions may require emergency unblinding: major bleeding, gastrointestinal (GI) hemorrhage, GI perforation, colitis, asthma and severe adverse events (SAEs), including heparin-induced thrombocytopenia, anaphylaxis, congenital abnormalities, persistent disabilities or disabilities that affect normal life functions, death or other conditions where the knowledge of the group allocation may be necessary to treat the subject. Severe adverse events will be defined as those that result in death, almost death, or when medical and/or surgical intervention are necessary to prevent death. Major bleeding will be defined as a decrease in hemoglobin of 2 g/dL or more, need for transfusion of two or more units of packed red blood cells or whole blood, or a critical site bleeding (intracranial, intra-spinal, intraocular, pericardial, intra-articular, intramuscular or retro-peritoneal). In case of a major bleeding event, emergency unblinding is warranted.

The request for unblinding will be sent to the principal investigator (PI). A 24-hour hotline will be available for situations when immediate medical attention is needed. A toll free number will be provided to the attending physician to proceed with code breaking. Trial interventions will be interrupted in such cases, but patient follow up will be maintained to provide data for intention to treat analysis. An independent medical monitor (IMM) will have access to the laboratory exams and may request unblinding, communicating with the treating physician, in cases of serious exam abnormalities, such as heparin-induced thrombocytopenia. Emergency unblinding will be reported in the emergency unblinding database, with reasons. The other participants and research personnel will remain blinded.

Eligibility Criteria

Pregnant women aged 18-40 (inclusive), before gestational week 10+0, with a past medical history of recurrent miscarriages defined as: two or more failed intrauterine clinical pregnancies as documented by ultrasonography or histopathologic examination, or three consecutive pregnancy losses, which are not required to be intrauterine, will be included.

Women with previous clinical diagnosis of one of the following forms of inherited thrombophilia: factor V Leiden mutation, prothrombin gene mutation (G20210A), protein S deficiency, protein C deficiency, or antithrombin deficiency.

Regarding exclusion criteria, recurrent pregnancy loss due to causes other than thrombophilia, diagnosis requiring anticoagulant treatment during pregnancy, such as prosthetic heart valves, history of venous thromboembolism, antiphospholipid syndrome, or others, use of other anticoagulants at the time of recruitment, simultaneous inclusion in other studies involving pharmacological interventions, past medical history of allergy or contraindications to LMWH or aspirin, past medical history of other hereditary diseases, past medical history of aspirin-induced asthma, past medical history of or current gastrointestinal (GI) illnesses, including ulcers, GI hemorrhage, GI perforation, and/or colitis, history of hepatic insufficiency, chronic kidney disease, congestive heart failure, physical or psychological inability to take part in the investigation, insufficient knowledge of the local language (unable to understand or write) hindering appropriate informed consent, previous active participants who experienced a miscarriage during the study and become pregnant again will not be available for study participation again, history of or current use of smoking, illegal and toxic substances.

Recruitment

Recruitment for this study will be done with three strategies: targeted/clinician based, since each clinical center involved will recruit for the trial through the identification of viable patients in collaboration with the local gynecological and hematological departments. Sites will email community and network physicians. Physicians will review eligibility criteria and refer interested patients to recruitment staff.

Will also apply broad-based recruitment/public awareness strategies as social media and hospital/clinical center advertising.

Finally, consortium partnership: National Organization for Rare Disorders/Thrombophilia Awareness Project, National Blood Clot Alliance, Women and Child Health Research Consortium.

Adherence

To optimize adherence, we will pursue the following steps: (i) at initial recruitment, patients will undergo a teaching session to learn adequate medication administration, and will receive a diary to document the medication intake regularly; (ii) at medication pickup (monthly in conjunction with scheduled OB/GYN visits) reminder sessions with a refreshment of the learned techniques will take place; (iii) a nurse study coordinator will check regularly via telemedicine (every other week) to identify problems with medication administration; (iv) subsequent sessions will occur at monthly follow-up visits, which will take place in coordination with OB/GYN appointments. Medication diaries will be reviewed, and participants will be asked about any problems they are having taking their study medication. There will be a brief discussion of reasons for missed doses and simple strategies for enhancing adherence, e.g., linking medication administration to meals or other daily activities. Participants will have an opportunity to ask questions and key messages from the initial session will be reviewed as needed; (v) with each visit, the nurse will collect blood samples, especially to assess anti-Xa levels and platelet counts. After that, the patient will visit the clinic monthly to collect her anticoagulant medication and for repeat blood work. The blood results will be available at all times to an IMM to ensure appropriate safety measures are enforced when needed. (vi) participants will be evaluated during their regular visits and flexible visits will be rescheduled when participants are unable to attend, call reminders will be set, comfortable and private areas will be available, and the importance of completion will be emphasized each visit.

Timeline

The schedule and procedures of the study are presented in Figure 1. The study participation is designed to be 44-48 weeks, depending on time of enrollment. Subjects will have monthly in-person study visits for exam and clinical assessments and biweekly telemedicine visits for safety monitoring. A full description of visit activities are described in Table 1 in Supplementary Appendix.

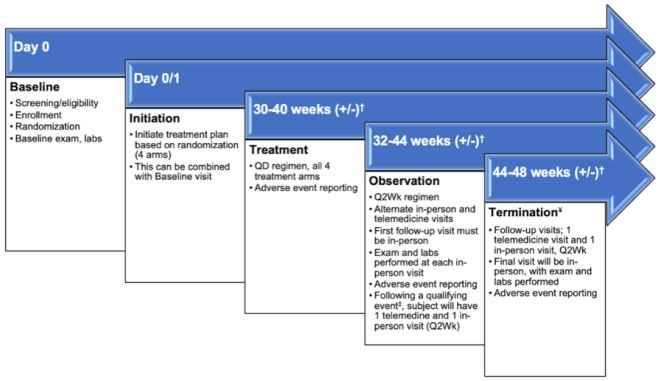

Figure 1. Participant Timeline.
†Duration is based on gestational age at enrollment (0-10 weeks), anticipated pregnancy term and adjustments due to event‡
‡Qualifying event is defined as live birth, miscarriage or VTE or major bleeding resulting in miscarriage. Subject will complete study termination¥ activities as a result.

Interventions

A nurse will show the subject how to administer the medication, and review the dosage and frequency, as follows (i) arm 1: enoxaparin 40 mg SC QD 06:00; standard low dose aspirin PO QD 06:00 after meal (81 mg US & Canada or 100 mg for all other participating countries); (ii) arm 2: Placebo enoxaparin SC QD at 06:00; standard low dose aspirin PO QD 06:00 after meal (81 mg US & Canada or 100 mg for all other participating countries); (iii) arm 3: Enoxaparin 40 mg SC QD at 06:00; placebo standard low dose aspirin PO QD 06:00 after meal; (iv) arm 4: Placebo enoxaparin SC QD at 06:00; placebo standard low dose aspirin PO QD 06:00 after meal.

Modification/discontinuation: potential side effects resulting from study participation include but are not limited to injection site irritation and GI upset.

Outcomes

Primary outcome: live birth (efficacy) - difference between the four treatment arms in the proportion of live birth during current pregnancy.

Secondary outcomes: treatment failure, composite outcome containing (miscarriage; stillbirth; maternal mortality; pre-eclampsia; placental abruption; intrauterine fetal growth restriction); safety (major bleeding events; venous thromboembolism event (VTE); heparin-induced thrombocytopenia (HIT); early postpartum hemorrhage; birth defects/malformations); laboratory (Anti-Xa activity, Antithrombotic properties, D-Dimer activity (ug/L), Prothrombin fragments (pmol/L), SPA in citrate whole blood).

Data management

An independent Data Monitoring Committee (DMC) of experts external to this study, without conflict of interest, will be established. The DMC will assess the progress, safety data and, if needed, critical efficacy endpoints. Confidentiality will be maintained during data monitoring, review and deliberations. Agendas of meetings will be developed by Project Officer (PO) and the DMC. Voting and minutes will be kept confidentially. Open Session and Close Section reports will be prepared, presented and appropriately distributed. Recommendations on continuity, pause or termination of the trial and necessary adjustments will be tracked to ensure prompt and effective implementation.

Interim Analysis

The data from the study will be monitored at 50% enrollment target. The interim analyses will be performed by an external statistical group, Quanticate©, blinded from the treatment allocation. The stopping boundary will be defined by the O'Brien-Fleming method.

Sample size calculation

Since the study design is a 2x2 factorial, the sample size calculation was conducted through estimated power analysis using a Cochran-Mantel-Haenszel (CMH) test. This approach allows minimizing the sample size, which would be larger throughout a typical sample size calculation. The test was carried out using Stata 17.0, and it takes the probabilities of success of the control group (placebo) in each stratum. This value was set as 0.5 and 0.6 based on the placebo response in the literature, for control groups for aspirin and heparin respectively (Kaandorp, 2010). Based on the preliminary and previous studies assumptions, the common odds ratio of the experimental group to the control group was established as 1.6 (Rai, 2000). We considered a power of 80% to demonstrate the superiority of heparin plus aspirin over placebo, at a 5% alpha. Thus, the sample size is 640, with 160 subjects per group. However, we expect 20% of dropouts, then the final sample size is set as 768 subjects (192 per group, study wide).

Statistical analysis for primary and secondary outcomes

Categorical variables will be analyzed by logistic regression analysis, controlling for strata (age ≥36 years and <36 years, previous miscarriages 2 and >2 and study sites). The results will be reported as odds ratio with 95% confidence interval. Statistical analysis for the comparison of mean values for continuous variables will be performed using a linear regression controlling for strata with post hoc multiple comparisons by Bonferroni correction. For subgroup analysis, logistic regression analysis will be applied to investigate for an interaction effect. Statistical analysis will be carried out using the Statistical Package of STATA Version 17.0 BE-Basic Edition (StataCorp LLC StataCorp 4905 Lakeway Drive College Station, Texas 77845 USA). A two-tailed P-value of <0.05 will be considered as statistically significant. Professional academic statisticians blinded to study groups will conduct all analyses. The outcome variables and all the corresponding planned statistical methods are described in Table 2 in Supplementary appendix.

Missing Data

All participants will be analyzed according to how they were originally randomized using intention to treat analysis (ITT). Data will be assumed missing at random (MAR), and analysis will be made using multiple imputations strategies. Markov Chain Montecarlo (MCMC) algorithm will be adopted. Sensitivity analysis with worst case analysis will be performed for missing data to determine the effects on the result.

Discussion

This trial, aims to test the efficacy and safety of the combination of enoxaparin and aspirin against each drug alone and placebo, to increase live birth in women with recurrent miscarriage and IT. In this case, factorial design allows the evaluation of the effect of two independent interventions (heparin and aspirin) using only one sample and increasing the efficiency (Fregni, 2018).There is only one study that focuses on the same target population, that is an ongoing multicenter two-armed, open-label randomized controlled trial, testing heparin against no treatment, in women with recurrent miscarriages and IT (de Jong, 2015).Clinical equipoise and relevance is ensured, given that there is no definitive evidence for the use of LMWH and aspirin in this condition. Standard medical guidelines do not recommend the use of active anticoagulants for this indication or, if so, are based solely on expert opinions and low level of evidence (Bates, 2012).

Limitations are posed by recruitment, which was a challenge noted in previous studies. Inherited thrombophilia in pregnant women is a rare condition and eligible patients may be unwilling to enroll given the potential randomization to a placebo group. Furthermore, screening for inherited thrombophilia is expensive and is not widely practiced or recommended outside of an experimental setting. A controversial aspect is the use of placebo in a pregnant population. This was deemed ethical and feasible, as well as methodologically important, based on previous studies showing a low rate of adverse events in the same population (Pasquier, 2015). Furthermore, all safety measures and trial oversight will be required, in order to safeguard and guarantee the best interests of the patients. Approval by the sites' ethical research committee and informed consent by the participants will be obtained.

Lastly, the evidence generated by this study will influence clinical practice, answering whether it is beneficial to apply these interventions for the population of women with inherited thrombophilia and recurrent miscarriages. We anticipate that this study will contribute to the current guidelines.

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