

APPLE

AntiPhospholipid syndrome low-molecular-weight heparin Pregnancy Loss Evaluation:
The pilot study

Protocol Title: A pilot study assessing the feasibility of a randomized controlled trial evaluating aspirin versus low-molecular-weight heparin (LMWH) and aspirin in women with antiphospholipid syndrome and pregnancy loss

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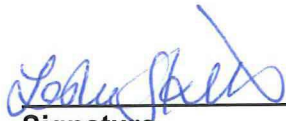
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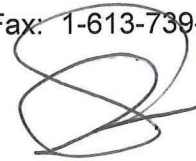
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PILOT STUDY SYNOPSIS

Title

A pilot study assessing the feasibility of a randomized controlled trial evaluating aspirin versus low-molecular-weight heparin (LMWH) and aspirin in women with antiphospholipid syndrome and pregnancy loss

Protocol Version

Version 4, November 7, 2017

Study Centres

The Ottawa Hospital, General Campus, Ottawa, ON
McMaster University Medical Centre, Hamilton, ON

Timeline

November 2017 - October 2019

Purpose of Pilot Trial

The purpose of this pilot trial is to determine the feasibility of conducting a multicenter randomized trial evaluating antepartum prophylaxis with aspirin versus LMWH/aspirin in women with confirmed APS and a history of late or recurrent early pregnancy loss.

Purpose of Full Trial:

The purpose of the full trial is to determine the efficacy and safety of antepartum prophylaxis with aspirin alone to improve the live birth rate in women with confirmed APS and a history of late or recurrent early pregnancy loss, compared to the standard of care regime of LMWH/aspirin prophylaxis.

Pilot Trial Endpoints

The primary feasibility outcome is the mean recruitment rate per center per month, calculated over 24 months. The secondary feasibility outcomes are: (defined in section 2.2)

1. Proportion of sites requiring >18 months to obtain all required approvals/contracts from time of delivery of all study documents
2. Proportion of screened patients who meet eligibility criteria
3. Proportion of eligible subjects who provide consent
4. Proportion of withdrawals/loss to follow-up among randomized patients
5. Proportion of subject crossover between study arms
6. Rate of compliance with antepartum LMWH use
7. Reasons for physician and patient non-consent

Full Trial Endpoints (Will be collected in the Pilot APPLE Trial)

The primary full trial outcome is the live birth rate. The secondary full trial outcomes are: (defined in section 2.3)

1. Livebirth rate according to central laboratory test results
2. Pregnancy loss by gestational age (<10 weeks, 10-19 weeks, ≥20 weeks)

3. Pre-eclampsia
 4. Early onset pre-eclampsia
 5. Severe pre-eclampsia
 6. Small-for-gestational age (SGA) <10th percentile
 7. SGA <5th percentile
 8. SGA <3rd percentile
 9. Placental abruption requiring delivery
 10. Pre-term delivery <37 weeks gestation
 11. Symptomatic venous thromboembolism (VTE)
 12. Symptomatic arterial thromboembolism (ATE)
 13. Major bleeding
 14. Clinically relevant non-major bleeding
 15. Peripartum major bleeding
 16. Peripartum minor bleeding
 17. Non-major non-CRNM bleeding
 18. Heparin induced thrombocytopenia (HIT)
 19. Symptomatic fracture
 20. Allergic reaction
 21. All-cause mortality
-

Trial Design/Methodology

The APPLE pilot trial is a feasibility study that is a multicentre, open-label, randomized controlled trial. Pregnant women with antiphospholipid syndrome (APS) and a history of late (≥ 10 weeks gestation) or recurrent early (2 <10 weeks) pregnancy loss will be recruited. Women with APS and a past history of venous or arterial thrombosis are not eligible for the trial (see below for the full list of exclusion criteria).

The study consists of four periods: screening, baseline and randomization, antenatal follow-up, labour and delivery, and post-partum follow-up.

Eligible and consenting subjects will be assigned to one of two study arms. Randomization is stratified by 'high-risk' or 'non-high risk' laboratory criteria and the timing of pregnancy loss (late loss or no late loss) (section 5.3):

Experimental arm

Open-label low-dose aspirin daily from randomization until delivery

Standard of care arm

Open-label LMWH prophylaxis until 37 weeks gestation **AND** low-dose aspirin daily until delivery.

The recommended "standard of care" arm is tinzaparin 4,500 IU sc daily until 20 weeks gestation, then tinzaparin 4,500 IU sc twice daily until 37 weeks gestation and aspirin 81 mg po daily until delivery. Treating physicians do have access to other LMWH (dalteparin, enoxaparin) as standard of care, but the suggested regime with tinzaparin is recommended. The use of LMWH prophylaxis beyond 37 weeks gestation and in the 6 week postpartum period will be left up to the discretion of the treating physician.

Each subject will receive education regarding LMWH administration, potential adverse effects and symptoms of VTE by clinic personnel (not research personnel), based on standard of care.

Visit Schedule

Screening, Baseline and Randomization

The subject will be evaluated for study eligibility by the investigator or delegated research personnel and once the consent has been assigned, a baseline assessment will be completed. Randomization is done at, or within 7 days of, the baseline visit. The feasibility outcomes will be collected using detailed screening and enrollment logs. Baseline blood work and an obstetrical ultrasound are recommended within 7 days of study enrolment.

Antenatal Follow-up

Subjects will attend a clinic visit at 20 ± 4 weeks gestation. All subjects will be monitored for study outcomes and adverse events (AE). Compliance of study medication will be measured at this visit.

Labour and Delivery

Study outcomes and AEs will be assessed through a review of subjects' medical records, which may include blood work, urinalysis and ultrasound reports. Data pertaining to the labour and delivery, as well as fetal health at birth will be documented.

Postpartum Follow-up

Subjects will attend a clinic visit at 6-12 weeks postpartum. Compliance of study arm during pregnancy will be measured at this visit. All subjects will be monitored for study outcomes and adverse events (AE).

Number of Subjects

The goal recruitment rate is recruitment of 1 subject every 2 months per centre, or a total of 24 patients in 24 months. The primary endpoint is the mean recruitment rate per month.

Study Population

Inclusion Criteria

Participants must be 18 years or older, have a confirmed pregnancy, and require at least one laboratory criteria and one clinical criteria, as listed below:

Clinical criteria: Past history of pregnancy loss defined as either:

- A. Two or more unexplained pregnancy loss before the 10th week of gestation
- B. One or more unexplained pregnancy loss at or beyond the 10th week of gestation

AND

Laboratory Criteria: At least one positive laboratory test listed below that is positive on two or more occasions at least 12 weeks apart*:

- A. Presence of lupus anticoagulant (LAC)

- B. Anticardiolipin antibody (aCL) of IgG or IgM with a titre > 99th percentile or > 40 GPL or MPL
- C. Anti- β 2 glycoprotein antibody of IgG or IgM with a titre > 99th percentile

*according to the diagnostic criteria of APS, diagnosis should be avoided if more than 5 years is separated between positive antibody testing and a clinical manifestation, such as pregnancy loss

Exclusion Criteria

1. Greater than 11 weeks +6 days gestational age at time of randomization
2. Indication(s) for prophylactic or therapeutic-dose anticoagulation
 - a. Venous thromboembolism (VTE)
 - b. Arterial thromboembolism (ATE)
 - c. Small-vessel thrombosis
 - d. Mechanical heart valve
 - e. Other indication for prophylactic or therapeutic-dose anticoagulation, at the discretion of the treating physician
3. Contraindication to heparin or aspirin including:
 - a. History of known heparin or LMWH allergy
 - b. History of known aspirin allergy
 - c. History of HIT
 - d. History of osteoporosis
 - e. Major bleeding within 7 days*.
 - f. Known thrombocytopenia, based on platelet count < 50 x 10⁹/L in the previous 3 months
 - g. Known hepatic disease, based on a medical history of elevated liver enzymes (3x ULN) and/or bilirubin (2xULN) documented within the last 3 months
 - h. Known renal failure, based on Creatinine Clearance < 30 mL/min (Cockcroft Gault) in the previous 3 months
 - i. Known severe hypertension, based on SBP > 200 mmHg and/or DBP > 120 mmHg in the previous 3 months
4. Received 7 or more doses of LMWH
5. Previous participation in the trial
6. Geographic inaccessibility**
7. Refused consent

*All other active or recent bleeding is at the discretion of the treating physician.

**At the discretion of the patient or the treating physician

Please note: Investigators should use caution when prescribing LMWH to patients with a history of gastric ulceration or whom weight >120kg or <45kg.

SCHEDULE OF MANDATORY EVENTS

	Screening, Baseline and Randomization	Antepartum: Week 20 ± 4 weeks visit	Labour & Delivery*	6-12 weeks Postpartum
Inclusion/Exclusion	√			
Consent	√			
Randomization	√			
Demographic	√			
Med/ObGyn Info/History	√			
Blood work ¹	√			
Dating ultrasound ²	√	√		
Concomitant Medication	√	√	√	√
Treatment Compliance		√		√
Drug Regime**		√		√
Labour & Delivery Data			√	
Infant Health			√	
Epidural Use				√
Outcome Information ³		√	√	√
Adverse Events ⁴		√	√	√

1 – BhCG is required to document current pregnancy, if not previously available. A complete blood count and creatinine will be drawn, if not previously available within the past 3 months. Lupus anticoagulant, anticardiolipin IgG and IgM antibodies and beta-2 glycoprotein IgG and IgM antibodies (if available) will be drawn to confirm eligibility if required, as well as bloodwork stored for central lab testing.

2 – Dating ultrasound results will be recorded, if available. An obstetrical ultrasound is recommended within 7 days of study enrolment as a baseline, starting at 6-7 weeks gestation.

3 – Outcome events will be recorded up to 6 weeks postpartum

4 – Adverse events requiring medical intervention will be followed until resolution or up to 90 days in which the AE will be considered chronic (at the discretion of the investigator).

* – Labour and delivery data will be gathered retrospectively by medical chart review. Any additional information and details required will be gathered by patient recall at the postpartum follow-up visit.

** – As applicable.

ABBREVIATIONS AND DEFINITIONS OF TERMS

AE	Adverse Event
ASA	Aspirin
APS	Antiphospholipid syndrome
AT	Antithrombin
bid	Twice Daily
BP	Blood Pressure
CBC	Complete Blood Count
DBP	Diastolic Blood Pressure
DVT	Deep Vein Thrombosis
IUGR	Intrauterine Growth Restriction
LMP	Last menstrual period
LMWH	Low Molecular Weight Heparin
o.d.	Once Daily
PE	Pulmonary Embolism
PIH	Pregnancy Induced Hypertension
RCT	Randomized Clinical Trial
RFTs	Renal function tests
SAE	Serious Adverse Event
SBP	Systolic Blood Pressure
SLE	Systemic Lupus Erythematosus
U/S	Ultrasound
ULN	Upper Limit of Normal
VTE	Venous Thromboembolic Event
Wk	Weeks

1. BACKGROUND

1.1 Why is a randomized clinical trial (RCT) needed?

Women with APS are at increased risk of pregnancy loss compared to the general population¹⁻³. The current standard of care, outlined in the CHEST guidelines, is to give antepartum prophylactic-dose LMWH and aspirin (ASA) to prevent future pregnancy loss⁴. Specifically, the CHEST guidelines mention “For women who fulfill the laboratory criteria for APLA syndrome and meet the clinical APLA criteria based on a history of three or more pregnancy losses, we recommend antepartum administration of prophylactic- or intermediate-dose UFH or prophylactic LMWH combined with low-dose aspirin, 75 to 100 mg/d, over no treatment”. In contrast, the Society of Obstetrics and Gynecology of Canada (SOGC) clinical practice guidelines recommend “Low-dose acetylsalicylic acid or low-dose acetylsalicylic acid plus low molecular weight heparin is recommended in pregnant women with confirmed antiphospholipid syndrome”

These guideline recommendations vary, and are based on trials with significant methodological flaws⁵⁻⁹. Three trials showed improved live birth rates with unfractionated heparin/ASA versus ASA alone⁵⁻⁷. Kutteh published a single-center non-randomized trial of 50 patients with at least 3 pregnancy losses and positive anticardiolipin antibodies, alternatively assigned to PTT-adjusted heparin or not⁵. There was a significant difference in live birth rate (80 versus 44%), however, the non-randomized study design significantly limits interpretation of these results⁵. Rai et al. completed a randomized trial of 90 patients with a history of 3 consecutive pregnancy losses and positive lupus anticoagulant or anticardiolipin antibodies (IgG > 5 GPL or IgM > 3 MPL) tested over 8 weeks apart, randomizing women to antepartum unfractionated heparin 5000 units sc Q12H and ASA versus ASA alone⁶. The live birth rate was improved, from 71 versus 42%⁶. Lastly, Goel et al. included a subset of 72 patients within a larger trial of 450 pregnant women with a history of 2 pregnancy losses and positive anticardiolipin antibodies⁷. Patients who received unfractionated heparin/ASA during pregnancy had improved live birth rate (84.8%) versus ASA alone (61.5%)⁷. Both trials were limited by small sample sizes and varying definitions of positive anticardiolipin antibodies.

In contrast, two subsequent trials evaluated LMWH/ASA versus ASA alone found no difference in live birth rates^{8,9}. Farquharson et al. randomized 98 women with at least 3 consecutive pregnancy losses or 2 pregnancy losses with proven fetal death after 10 weeks gestation with persistently positive lupus anticoagulant and/or anticardiolipin antibodies at least 6 weeks apart⁸. Women were randomized to antepartum LMWH/ASA versus ASA alone until delivery. There was no difference in live birth rate between treatment arms (78% versus 72%, p=0.49)⁸. One limitation to this study is a 25% reported crossover rate between study arms, based on physician or patient preference. Lastly, Laskin et al. completed a randomized trial of women a history of 2 or more pregnancy losses, including patients with one of: ANA positivity, lupus anticoagulant or anticardiolipin antibodies, or inherited thrombophilia, randomizing participants to LMWH/ASA versus ASA alone during pregnancy⁹. Of the subgroup with lupus anticoagulant or anticardiolipin antibody positivity, there was no difference in the live birth rate (77% versus 75%) among 42 patients⁹. Laskin et al. conclude “these findings contribute to the growing body of literature that contests the emerging standard of care comprising LMWH/ASA”. Limitations to these negative studies include a 25% crossover rate between study arms⁸, small sample size and

varying definitions of APS^{8,9}. Furthermore, no trial has evaluated prophylaxis in women with pregnancy loss and persistent anti-β2 glycoprotein antibodies. Given the poor quality evidence and mixed results for the use of LMWH/ASA prophylaxis in this setting, a large randomized trial is still needed.

When uncertainty exists in pregnancy, health care providers and patients often err on the side of treatment in hopes of preventing complications. Our goal is to minimize uncertainty, to provide clear recommendation to women with APS and pregnancy loss based on high-quality evidence. The full APPLE trial will compare the live birth rates in women assigned to the standard of care arm, prophylactic LMWH and ASA, compared to the experimental arm, ASA alone.

1.2 Why is a clinical trial needed now?

Data from the TIPPS trial and two meta-analyses have recently shown that LMWH likely does not prevent pregnancy loss or complications in women with or without inherited thrombophilia¹⁰⁻¹². Given the poor-quality of existing evidence, evaluation of LMWH/ASA prophylaxis is still needed in APS patients⁴⁻⁹. We conducted an online survey of 109 CanVECTOR Network and Thrombosis Canada physician members, 22 (20%) responded, and 91% would hypothetically approach a patient for enrollment in our trial, in either all (68%) or a subset (23%) of APS patients.

1.3 What are the risks with the standard of care regime of prophylactic LMWH/ASA?

Downsides of LMWH include burden of injections (up to 400 injections/pregnancy), cost (\$4,000/pregnancy) and side effects of LMWH. In a systematic review of LMWH use in pregnancy, the most common side effects were bleeding (1.98%) which included significant antepartum bleeding, postpartum hemorrhage, and abdominal hematomas¹³. Allergic skin reactions (1.8%) were also reported, some of which may be associated with HIT antibodies¹³. Being on heparin lowers the likelihood that a woman receives epidural analgesia at the time of delivery, given the documented risk of epidural hematoma and associated hemiplegia when LMWH is not held prior to epidural analgesia¹⁴⁻¹⁶. Serious and life-threatening complications of LMWH are rare, but possible. HIT has rarely been reported in pregnancy with the use of LMWH^{17,18}. The risk of osteoporosis and symptomatic fracture is rare, and significantly lower compared to unfractionated heparin^{19,20}.

1.4 What are the risks with the experimental arm (ASA alone)?

As outlined in Section 1.0, given the poor quality of evidence in the LMWH/ASA arm, further evaluation of LMWH/ASA versus ASA is still needed. Based on data from meta-analyses and large randomized controlled trials, aspirin has no excess bleeding risk or neonatal complications in other populations of pregnant women, such as those with pre-eclampsia and women with prior pregnancy loss with or without inherited thrombophilia. There may be a possible small increased risk of gastroschisis with early exposure to aspirin²¹⁻²³.

A small randomized double-blind placebo controlled trial of 50 women with a history of recurrent pregnancy loss and positive antiphospholipid antibodies showed no difference in live birth rate or neonatal complications when treated with aspirin alone versus placebo during pregnancy²⁴. While a 'no treatment' arm could be considered for the experimental arm, there was a low proportion (23%) of physician acceptability of a no ASA arm in a national survey of CanVECTOR and Thrombosis Canada

members. Given the established safety profile of ASA in pregnancy and physician acceptability, ASA alone was chosen as the experimental arm.

1.5 Why is a pilot RCT needed before a full RCT can be completed?

Because the use of antepartum prophylactic-dose LMWH and aspirin have become the standard of care in women with APS and past pregnancy loss, and is recommended in clinical practice guidelines⁴, the recruitment of subjects into a randomized clinical trial may be challenging. Therefore, a pilot trial to assess feasibility is first needed.

1.6 Need for an open-label trial

Because of initial poor recruitment in the TIPPS trial that evaluated LMWH in a double-blinded fashion (24 women enrolled after 3 years), the design of that trial was changed to open-label use of LMWH, which subsequently led to improved recruitment rates¹¹. Similarly, the pilot trial APPLE will use an open-label trial design to increase eligible women's acceptance of the trial and eliminate the burden of placebo injections. The primary endpoint in the larger multi-center trial is the live birth rate, which is objective and, therefore, not expected to change with the open-label use of LMWH.

1.7 Need for 'pragmatic' use of LMWH

The use of prophylactic-dose LMWH and ASA has become the standard of care to prevent future pregnancy loss in women with APS, despite limited evidence for their use. Our pilot trial is looking at the feasibility of removing LMWH use in the antepartum setting, by comparing LMWH/ASA versus ASA alone. We recommend a "standard of care" regime of tinzaparin 4,500 IU sc daily until 20 weeks gestation, then tinzaparin 4,500 IU sc twice daily until 37 weeks gestation and aspirin 81 mg po daily until delivery. Treating physicians do have access to other LMWHs (dalteparin, enoxaparin) as standard of care, but this suggested regime with tinzaparin is recommended for consistency.

There is pharmacokinetic data in pregnant patients to suggest an increased dose after 20 weeks gestation is needed to maintain anti-Xa levels in the reference range, based on increased renal clearance later in pregnancy²⁶. While the recommended LMWH regime includes tinzaparin²⁷, pharmacokinetic data exists for all available LMWHs (dalteparin²⁵, enoxaparin²⁸).

1.8 Why are women with 2 losses included?

The Sapporo Criteria definition of APS is based on a pregnancy loss definition of 1 late loss at ≥ 10 weeks gestation, or 3 recurrent early losses < 10 weeks gestation²⁹. However, women are often referred for a question of thromboprophylaxis after 2 early pregnancy losses and with little evidence to guide therapy, it is not uncommon for physicians to offer antepartum LMWH/ASA prophylaxis after 2 losses. Knowing if ASA is as good as ASA/LMWH in women with 2 losses would be an important finding. The APPLE pilot trial will include women with 2 or more early pregnancy losses, in order to provide data for this important subgroup of women.

2. TRIAL OBJECTIVES

2.1 Trial Purpose

The purpose of this pilot trial is to determine the feasibility of conducting a multicenter randomized trial evaluating antepartum prophylaxis with ASA versus LMWH/ASA in women with confirmed APS and a history of late or recurrent early pregnancy loss.

Given the large sample size needed to adequately power a large multicenter trial that assesses the efficacy of ASA alone versus LMWH/ASA (section 8.2), we first need to determine if we can meet minimum recruitment rates needed for a full multicenter trial to be possible. To test this, we will open the trial at 2 centers with the goal of recruiting one patient every two months. The randomization design for the pilot feasibility trial is the same as the full trial, to best represent what actual recruitment rates and trial conditions would be like in the full trial. If the pilot feasibility trial is successful, then the secondary outcomes collected will be used in the analysis of the larger full multicenter trial.

The purpose of the full trial will be to determine the efficacy and safety of antepartum prophylaxis with aspirin alone to improve the live birth rate in women with confirmed APS and a history of late or recurrent early pregnancy loss, compared to the standard of care regime of LMWH/ASA prophylaxis.

2.2 Pilot Trial Objectives

The primary objective of the pilot trial is the mean recruitment rate per center per month, calculated over 24 months.

Secondary objectives of the pilot trial will include evaluating:

- The feasibility of obtaining all required approvals/contracts in a timely fashion
- The number of patients who are ineligible based on the exclusion criteria
- The consent rate for eligible women who are approached
- The crossover rate between study arms
- The compliance rate of antepartum LMWH use
- Explore the reasons for non-consent among physician and patients

2.3 Pilot Trial Endpoints

The primary feasibility outcome of the pilot trial is a mean recruitment rate of 0.5 subjects per center per month. The secondary feasibility outcomes of the pilot trial and associated *a priori* feasibility targets are as follows:

1. Proportion of sites requiring >18 months to obtain all required approvals/contracts from time of delivery of all study documents: <50%.
2. Proportion of screened patients who meet eligibility criteria (i.e. patients who meet inclusion criteria and are also eligible based on exclusion criteria): >30% of subjects
3. Proportion of eligible subjects who provide consent: >30% of subjects
4. Proportion of withdrawals/loss to follow-up among randomized patients: <10% of subjects
5. Crossover rate between standard of care and experimental study arms: <20%
6. Level of compliance with study drug: >80% compliance rate of total antepartum LMWH use
7. Reasons for physician and patient non-consent

2.4 Full Trial Objectives

The primary objective of the full trial will be to determine the efficacy of ASA in improving live birth rate among women with APS and past pregnancy loss, compared to the standard of care arm of LMWH/ASA.

Secondary objectives of the full trial will include assessing the efficacy of ASA to prevent other pregnancy complications (pre-eclampsia, SGA, placental abruption, pre-term labor) and thrombotic complications (VTE, death from VTE, ATE) in women with APS and past pregnancy loss, versus LMWH/ASA. Safety data will also be reported, including bleeding and other adverse events.

2.5 Full Trial Endpoints

The full trial endpoints will also be collected in the Pilot APPLE Trial. The primary full trial outcome is the livebirth rate. The secondary full trial outcomes are:

1. Livebirth rate according to central laboratory test results
2. Pregnancy loss by gestational age (<10 weeks, 10-19 weeks, ≥20 weeks)
3. Pre-eclampsia
4. Early onset pre-eclampsia
5. Severe pre-eclampsia
6. Small-for-gestational age (SGA) <10th percentile
7. SGA <5th percentile
8. SGA <3rd percentile
9. Placental abruption requiring delivery
10. Pre-term delivery <37 weeks gestation
11. Symptomatic venous thromboembolism (VTE)
12. Symptomatic arterial thromboembolism (ATE)
13. Major bleeding
14. Clinically relevant non-major bleeding
15. Peripartum major bleeding
16. Peripartum minor bleeding
17. Non-major non-CRNM bleeding
18. Heparin induced thrombocytopenia (HIT)
19. Symptomatic fracture
20. Allergic reaction
21. All-cause mortality

2.6 Endpoint Definitions

Livebirth

A birth in which a baby is born alive. If there are multiple births (i.e. twins) then it is counted as one live birth if at least one is born alive.

Pregnancy loss by gestational age

Will record the pregnancy loss based on the gestational age in weeks. Will use the best information available to determine the gestational age at the time of the pregnancy loss. First, pathological examination of products of conception then ultrasound dating assessment at the time of pregnancy loss then medical documentation of timing of pregnancy loss then patient recall of timing of pregnancy loss. A pregnancy loss of one or more multiple births (i.e. twins) will be recorded.

Pre-eclampsia

Systolic blood pressure \geq 140 mm Hg or diastolic blood pressure \geq 90 mm Hg and proteinuria >0.3 g/ 24 hours or \geq 30 mg/mmol urinary creatinine in a spot (random) urine sample.

- a. Early onset pre-eclampsia: Pre-eclampsia $<$ 34 weeks gestation
- b. Severe pre-eclampsia: At least one criterion: systolic blood pressure \geq 160 mm Hg or diastolic blood pressure \geq 110 mm Hg, proteinuria $>$ 5 g/24 hours, AST, ALT, or bilirubin greater than 2xULN, platelets $<$ 100 x 10⁹ /L, pulmonary edema, seizures (eclampsia), headache or other neurological manifestations (stroke, intracranial hemorrhage, cerebral edema, hyperreflexia and visual impairment), coagulopathy (elevated INR/PT or PTT greater than 1.5xULN or fibrinogen $<$ 2 g/L), oliguria ($<$ 30 mL/hour for 3 hours), or HELLP syndrome. HELLP syndrome requires 3 criteria: hemolysis [lactate dehydrogenase (LDH) $>$ 600 IU/L or serum bilirubin $>$ 1.2 mg/dl] an abnormal elevation of liver enzymes (AST or ALT greater than 2xULN) and platelets $<$ 100 x10⁹ /L.

Small-for-gestational age (SGA)

Percentile of birth weight determined using local gender and gestational age specific birth weight charts.

- a. SGA: $<$ 10th birth weight determined using local gender and gestational age specific birth weight charts.
- b. Severe SGA: $<$ 5th percentile of birth weight determined using local gender and gestational age specific birth weight charts.
- c. Very Severe SGA: $<$ 3rd percentile of birth weight determined using local gender and gestational age specific birth weight charts.

Placental abruption

Clinical diagnosis of placental abruption leading to delivery.

Pre-term delivery

Delivery at $<$ 37 weeks gestation.

Symptomatic VTE

VTE is defined as symptomatic deep vein thrombosis (DVT) or pulmonary embolism (PE). DVT is defined as a non-compressible segment or filling defect proximal to the calf trifurcation based on ultrasonography, venography or magnetic resonance imaging. PE is diagnosed by a new intraluminal filling defect on CT pulmonary angiogram (segmental artery or more proximal), a high probability perfusion defect on a ventilation-perfusion study based on PLOPED criteria with planar images, or PE as a definite or probably cause of death.

Symptomatic ATE

Symptomatic arterial events include ischemic stroke, transient ischemic attack, myocardial infarction, peripheral embolism, or death due to a vascular cause based on autopsy findings (excluding PE or intracranial hemorrhage).

Major bleeding

Clinical or radiographical evidence of bleeding associated with one of: with a fall in hemoglobin of 20 g/L (2 g/dL) 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.

Clinically relevant non-major bleeding (CRNMB)

Any sign or symptom of hemorrhage that does not fit into the definition of major bleeding, peri-partum major bleeding, postpartum major bleeding, or peri-partum minor bleeding, and meets at least one of the following criteria: requiring medical intervention by a health care professional, leading to hospitalization or increased level of care, prompting a face to face (not just telephone or electronic communication) evaluation. CRNMB will be sub-categorized into the antepartum and postpartum (24 hours-6 weeks postpartum) period.

Non-major non-CRNM bleeding

All other bleeding that does not fit into major bleeding, CRNMB, or peripartum bleeding.

Peripartum major bleeding

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 40 g/L (4 g/dL) 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

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.

HIT

Heparin-induced thrombocytopenia (HIT) is defined as a clinical diagnosis of HIT (based on a 4T score that is intermediate or high probability) and a minimum of a positive PF4 HIT ELISA assay.

Symptomatic Fracture

A symptomatic fracture confirmed by radiography.

Allergic reaction

A reaction following the administration of LMWH that results in anaphylaxis or a rash requiring discontinuation of the allocated LMWH.

All-cause mortality

The cause of death will be based on all available information, including clinical documents, autopsy if available, and official death certificate.

3. TRIAL DESIGN

3.1 Trial Design

APPLE is a feasibility pilot, open-label, randomized controlled clinical trial in 2 centers, Ottawa and Hamilton, Ontario. Pregnant women with antiphospholipid

syndrome and past late (≥ 10 weeks gestation) or recurrent early ($2 < 10$ weeks) pregnancy loss will be randomized to low-dose ASA daily (experimental arm) or prophylactic-dose LMWH/ASA (standard of care arm). Other than LMWH use, the two arms will have identical follow-up care.

The study consists of four periods: screening and randomization, antenatal follow-up, labour and delivery, and post-partum follow-up. Maximum time on study will vary between 34 and 52 weeks.

3.2 Screening, Baseline and Randomization

Feasibility outcomes will be collected using detailed screening and enrollment logs. Screening logs will document reasons for ineligibility. We will also explore and document reasons for non-consent from patients and local investigators in order to refine the larger multicenter study.

Randomization is done within 7 days of completing the baseline assessment. Subjects who meet the inclusion/exclusion criteria and have signed informed consent will be assigned to one of the two treatment groups (ASA vs LMWH/ASA), stratified by pregnancy loss history and laboratory testing. Details on how a subject is randomized are provided in section 5.3.

Prior to randomization and study entry, a BHCG is needed to confirm the current pregnancy, if not already available. CBC and creatinine blood work will be drawn at baseline, if not previously available within the past 3 months. Past obstetrical history will be recorded.

Subjects can still be enrolled and randomized in the study if they only have one positive antiphospholipid antibody result. A second set of labs would then be drawn 12 weeks apart from the first set, and if the second set is positive they will continue in the study. If the second set is negative, the subject will be withdrawn from the study.

Within 7 days of enrollment, a baseline obstetrical ultrasound is recommended. If the subject is enrolled in early first trimester (< 6 weeks gestation) then the ultrasound will be completed at 6-7 weeks gestation. Blood work will also be drawn on enrollment and stored to be tested in a central laboratory (lupus anticoagulant, anticardiolipin antibody, beta-2 glycoprotein antibody).

Each subject will receive education regarding LMWH administration, potential adverse effects and symptoms of VTE by clinic personnel (not research personnel), based on standard of care.

3.3 Antenatal Follow-Up

The antenatal follow-up visit will take place at 20 ± 4 weeks gestation. Study outcomes and/or adverse events will be assessed and documented. If available, obstetrical ultrasound results will be recorded.

The subject will be asked to bring the Patient Medication Diary to the follow-up visit. Using subject recall and the Medication Diary, treatment compliance will be documented.

For subjects randomized to LMWH/ASA, the suggested protocol is tinzaparin 4,500 IU sc daily until 20 weeks gestation, then tinzaparin 4,500 IU sc twice daily until 37 weeks gestation and ASA 81 mg po daily until delivery.

Use of trial medication beyond 37 weeks gestation is left up to the discretion of the treating physician. Discontinuing the study medication at 37 weeks will minimize the possibility of being anticoagulated at the time of delivery or epidural analgesia.

3.4 Labour and Delivery

Subjects randomized to receive LMWH/ASA will be instructed to discontinue LMWH at 37 weeks, or at the onset of labour if they have not already done so.

For all subjects, information regarding labour, anaesthesia, delivery, complications, fetal weight and health at birth will be recorded. Outcomes of interest and adverse events will be recorded based on available hospital records.

Subjects who prefer to deliver at another hospital or at home, may participate in the study, so long as arrangements are made to collect the labour and delivery data retrospectively.

3.5 Postpartum Follow-up

The use of postpartum LMWH and ASA prophylaxis will be left up to the treating physician, and will be documented. At the postpartum visit at 6-12 weeks postpartum, outcome events will be recorded up until 6 weeks postpartum. Adverse events requiring medical intervention will be followed until resolution or will be classified as chronic/ongoing after 90 days at the discretion of the investigator.

3.6 End of Study

Study crossover, compliance, and early termination will be documented. Reasons for study crossover (patient or physician preference) will be documented. If a subject terminates the study early, ASA and/or LMWH will be left to the treating physician, and documented. Primary and secondary outcomes will be captured.

4. SELECTION AND WITHDRAWAL OF SUBJECTS

4.1 Inclusion Criteria

All of the following inclusion criteria must be met to be eligible: 1) confirmed pregnancy, 2) 18 years or older, 3) one or more clinical criteria as outlined below, 3) one or more laboratory criteria as outlined below, and 4) written informed consent.

Clinical criteria: Past history of pregnancy loss defined as either:

- A. Two or more unexplained* pregnancy loss before the 10th week of gestation**
- B. One or more unexplained* pregnancy loss at or beyond the 10th week of gestation**

AND

Laboratory Criteria: At least **one** positive laboratory test listed below that is positive on **two** or more occasions at least 12 weeks apart***:

- A. Presence of lupus anticoagulant (LAC)

- B. Anticardiolipin antibody (aCL) of IgG or IgM with a titre > 99th percentile or > 40 GPL or MPL
- C. Anti- β 2 glycoprotein antibody of IgG and/or IgM with a titre > 99th percentile

Subjects can initially be eligible for enrollment and randomization if they only have one positive lab result. A second set of labs would be drawn 12 weeks apart from the first lab result, and if the second set is positive they will continue in the study. If the second set is negative, the subject will be withdrawn from the study.

*Examples of explained miscarriage or fetal loss include: loss associated with severe congenital malformations, chromosomal abnormalities, neonatal alloimmune haemolytic anaemia, recent CMV infection, positive fetal or placental Listeria cultures, and women with known abnormal uterine anatomy.

**Determining timing of pregnancy loss: in reviewing the past obstetrical history the investigator will use the best available information (First, pathological examination of products of conception then ultrasound dating assessment at the time of pregnancy loss then medical documentation of timing of pregnancy loss then patient recall of timing of pregnancy loss) to determine the gestational age at the time of pregnancy loss.

***According to the diagnostic criteria of APS, diagnosis should be avoided if more than 5 years is separated between positive antibody testing and a clinical manifestation, such as pregnancy loss

4.2. Exclusion Criteria

1. Greater than 11 weeks +6 days gestational age at time of randomization
2. Indication(s) for prophylactic or therapeutic-dose anticoagulation
 - a. Venous thromboembolism (VTE)
 - b. Arterial thromboembolism (ATE)
 - c. Small-vessel thrombosis
 - d. Mechanical heart valve
 - e. Other indication for prophylactic or therapeutic-dose anticoagulation, at the discretion of the treating physician
3. Contraindication to heparin or aspirin including:
 - a. History of known heparin or LMWH allergy
 - b. History of known aspirin allergy
 - c. History of HIT
 - d. History of osteoporosis
 - e. Major bleeding within 7 days*.
 - f. Known thrombocytopenia, based on platelet count < 50 x 10⁹/L in the previous 3 months
 - g. Known hepatic disease, based on a medical history of elevated liver enzymes (3x ULN) and/or bilirubin (2xULN) documented within the last 3 months
 - h. Known renal failure, based on Creatinine Clearance < 30 mL/min (Cockcroft Gault) in the previous 3 months
 - i. Known severe hypertension, based on SBP > 200 mmHg and/or DBP > 120 mmHg in the previous 3 months
4. Received 7 or more doses of LMWH
5. Previous participation in the trial

6. Geographic inaccessibility**
7. Refused consent

*All other active or recent bleeding is at the discretion of the treating physician.

**At the discretion of the patient or the treating physician

Note: Subjects currently receiving aspirin are eligible for the study.

Please note: Investigators should use caution when prescribing LMWH to patients with a history of gastric ulceration or whom weight >120kg or <45kg.

4.3 Withdrawal of Subjects

A subject has completed the study once all follow-up procedures have been completed.

Study drug is discontinued and appropriate clinical management is initiated in the case of the following outcome events (as defined in section 2.6). For the subjects who reach a study endpoint, LMWH and aspirin prophylaxis will be left up to the discretion of the treating physician, and documented.

1. Pregnancy loss
2. Pre-eclampsia
3. Placental abruption
4. Preterm delivery (<37 weeks)
5. VTE (objectively documented DVT, PE or sudden death)
6. Major bleeding
7. Thrombocytopenia (Platelet count <50 x 10⁹/L)
8. Symptomatic fracture

In the event that the outcome does not result in infant delivery (ie. diagnosis of a VTE, fracture, thrombocytopenia, major bleed), antenatal follow-up will continue as scheduled in the protocol and data will be collected until 6 weeks post-partum or pregnancy related outcome.

A subject can be withdrawn from the trial treatment if, in the opinion of the investigator, it is medically necessary. This does not always correspond to study withdrawal – in which case antenatal follow-up will continue as scheduled in the protocol and data will be collected until the 6-12 weeks post-partum clinic visit, up until 6 weeks postpartum.

If the subject is prematurely discontinued from participation in the study, the study personnel will make every effort to obtain, and record, information about the reasons for discontinuation and any adverse events and if possible perform all safety assessments.

A subject may voluntarily withdraw participation in this study at any time, including for future data collection. If the subject does not return for a scheduled visit, every effort will be made to contact the subject. In any circumstance, every effort will be made to document the reason for withdrawal and when possible all safety assessments will be done.

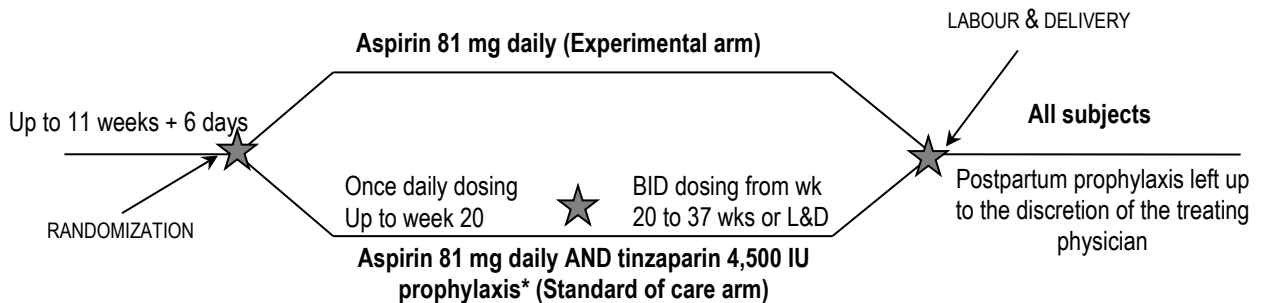
All data will be reported for any subject randomized and not completing the study. Subjects withdrawn as a result of an adverse event thought to be related to the study drug will not be replaced. All safety data collected from all subjects during the study will be analyzed.

5. RECRUITMENT

5.1 Treatment Schedule

Upon enrolment into the study, subjects will be randomized to receive low dose aspirin alone until delivery, or low-dose ASA (until delivery) and prophylactic-dose LMWH until 37 weeks gestation or the onset of labour. The LMWH regime will be at the discretion of the treating physician, with a suggested regime as follows: tinzaparin 4,500 IU sc daily until 20 weeks gestation, and then 4,500 IU sc twice daily until 37 weeks gestation. Treating physicians will have access to other LMWHs as alternative 'standard of care' options, however, one regime is recommended for consistency (Figure 1). Dosing frequency after 20 weeks gestation is at the discretion of the treating physician based on practice-pattern differences, however, a twice daily dosing is recommended. The use of daily tinzaparin in the 6 week postpartum period is left to the discretion of the treating physician. Concomitant NSAID use is not recommended.

Figure 1. Recommended LMWH protocol



*Alternatives include dalteparin 5,000 IU or enoxaparin 40 mg, however, tinzaparin 4,500 IU is preferred when possible.

Each subject will receive education regarding LMWH administration, potential adverse effects and symptoms of VTE by clinic personnel (not research personnel), based on standard of care.

5.2 Randomization

Consenting subjects will be randomized up to 7 days after baseline visit. Treatment allocation is determined by central web-randomization. A randomization list has been generated by the trial statistician prior to embarking on the trial. Randomization is in permuted blocks of 10, prepared using random number tables, and is stratified according to laboratory criteria ('High-risk' versus 'Non-high risk' laboratory groups, defined below) and the timing of pregnancy loss (late loss versus no late loss) Any past history of loss ≥ 10 weeks gestation will be included in the late loss group.

Definitions of groups:

No Late Loss: Defined as only unexplained pregnancy loss(es) less than 10 weeks gestation

Late loss: Defined as one or more unexplained pregnancy loss(es) at or beyond the 10th week of gestation

‘Non-high risk’ laboratory results are defined by both of:

- 1) aCL of IgG and/or IgM positivity (>99th percentile) \leq 40 GPL or MPL
- 2) No ‘High risk’ laboratory results present

‘High risk’ laboratory results are defined by the presence of at least ONE of:

- 1) Lupus anticoagulant present
- 2) aCL of IgG and/or IgM with a titre > 40 GPL or MPL
- 3) Anti- β 2 glycoprotein antibody of IgG and/or IgM with a titre > 99th percentile

Randomization groups:

Non-high risk and no late loss: Group A

Non-high risk and late loss: Group B

High risk and no late loss: Group C

High risk and late loss: Group D

5.3 Web Base Randomization

The randomization process is started by the local study personnel (investigator or study coordinator) who initiates a randomization request by accessing the APPLE webpage and randomization request form. This portion of the webpage requires authorised users to enter a username and password. To randomize a subject the following information is required: category of ‘high’ versus ‘non-high’ risk laboratory criteria, and category of early versus late pregnancy loss. The program will request that the information entered be verified for accuracy and will prompt any errors or missing fields to be completed. When the information is accepted, the program will provide the delegated study coordinators, Qualified Investigator, and pharmacy team the treatment allocation by using the next allocation from that block.

5.4 Study drug management

The study drug (ASA and LMWH) will be dispensed by the local site hospital Pharmacy Department for all participants in both treatment arms. The local site hospital Pharmacy Department will be responsible for labeling, handling, and dispensing of the study drug.

6. MEASUREMENTS AND EVALUATIONS

6.1 Demographic and Baseline Characteristics (Baseline Period)

Subjects will be required to sign a consent form prior to any study procedures being undertaken. During the baseline period, before randomization (up to 11 weeks + 6 days gestation), the following information will be measured and/or recorded:

- Demographic data:
 - Age and birth month
 - Race/Ethnicity

- Height and weight prior to pregnancy
- Current pregnancy data:
 - Gestational age/estimated date of delivery
 - Type of test used to confirm pregnancy and date performed
 - Method of conception
 - Number of fetuses
- Medical history:
 - Presence of SLE diagnosed by rheumatologist or internist
 - Presence of livedo reticularis
 - Presence of hypertension
 - Presence of type 1 diabetes
 - Presence of type 2 diabetes
 - Smoking status
 - Known inherited thrombophilia present and details
- Obstetrical History:
 - Age at start of each pregnancy
 - Gravida, term deliveries, premature deliveries, spontaneous abortions, therapeutic abortions, living children and multiple pregnancies
 - Number, timing and reason of past pregnancy losses
 - According to < 10 weeks, ≥ 10 weeks
 - Past pregnancy complications
 - Pre-eclampsia, SGA, placental abruption, pre-term delivery
 - Treatment of past pregnancies (use of LMWH and/or ASA)

6.2 Laboratory and Imaging Assessments

A baseline BHCG will be drawn at the screening period, if not already available for the current pregnancy. A CBC and creatinine will be drawn at baseline, if not previously available within the past 3 months. A second set of antiphospholipid labs can be drawn if required to meet the eligibility laboratory criteria, 12 weeks apart from the first set. The subject can be enrolled and randomized to a treatment arm prior to receiving the second set of lab results. According to the diagnostic criteria of APS, diagnosis should be avoided if more than 5 years is separated between positive antibody testing and a clinical manifestation, such as pregnancy loss.

Within 7 days of enrollment, a baseline obstetrical ultrasound is recommended. If the subject is enrolled in early first trimester (<6 weeks gestation) then the ultrasound will be completed at 6-7 weeks gestation. Blood work will also be drawn on enrollment and stored and tested in a central laboratory (lupus anticoagulant, anticardiolipin IgG and IgM antibodies, beta-2 glycoprotein IgG and IgM antibodies).

Additional blood work or imaging assessments will be ordered if an outcome is clinically suspected, and will be left up to the discretion of the treating physician.

6.3 Prior and Concomitant Therapy

Concomitant therapy will be at the discretion of the primary physician. Selected concomitant medications will be documented on the case report forms. Investigators who decide to discontinue antenatal LMWH at 37 weeks gestation and initiate the use of unfractionated heparin will be required to document this on the concomitant medication record in the case report form. NSAID use is not recommended.

6.4 Antenatal Follow-up

Antenatal follow-up will occur at 20 (\pm 4) weeks gestation and the following information will be recorded:

- Gestational age
- Medication regime plan
- Medication compliance
- Possible adverse events and outcomes

6.5 Postpartum Follow-up

Postpartum follow-up will occur at 6-12 weeks postpartum and the following information will be recorded:

- Number of weeks postpartum at time of visit
- Postpartum medication regime plan (if applicable)
- Epidural use during labour
- Medication compliance
- Possible adverse events and outcomes

6.6 Labour and Delivery Record

Available labor and delivery information will be recorded:

- Date of delivery
- Gestational age at delivery
- Mode of delivery
 - Vaginal delivery versus caesarian section, including reason for C-section (scheduled versus unscheduled/emergency)
- Estimated blood loss in mL, if available
- Spontaneous versus induction of labor, and reason for induction if available
- Live birth
- Infant data
 - Sex, weight and percentile
 - NICU admission
 - Infant survival (record cause if known)

6.7 Adjudication

A blinded adjudication committee will be assembled to review the following suspected outcomes: pre-eclampsia, placental abruption, symptomatic VTE, symptomatic ATE, suspected bleeding, suspected HIT and/or death.

Each suspected event will be reviewed by two committee members to determine if it meets the criteria for the full trial outcomes as outlined in the protocol. If the two reviewers disagree, a third member will review to act as a tie-breaker.

All relevant medical records, imaging results, etc. will be collected to prepare an adjudication report.

6.8 Adverse Events

AEs will be elicited, monitored and recorded throughout the study. For each subject, AEs will be submitted to the trial office following visits at gestational week 20 \pm 4 weeks, labour and delivery, and 6-12 weeks postpartum.

7. ADVERSE EVENTS

7.1 Definition

An **adverse event (AE)** is any untoward medical occurrence in a subject or trial subject administered a drug or biologic (medicinal product) or using a medical device; the event does not necessarily have a causal relationship with that treatment or usage.

AEs include the following:

- All suspected adverse medication reactions.
- All reactions from medication abuse, withdrawal, sensitivity, or toxicity.
- Apparently unrelated illnesses, including the worsening of a pre-existing illness (definition below).
- Injury or accidents.
 - **Note** that if a medical condition is known to have caused the injury or accident (e.g., a fall secondary to dizziness), the medical condition (dizziness) and the accident (fall) will be reported as two separate AEs.
- Abnormalities in physiological testing or physical examination (findings that require clinical intervention or further investigation beyond ordering a repeat [confirmatory] test).
- Laboratory abnormalities that require clinical intervention or further investigation (beyond ordering a repeat [confirmatory] test) unless they are associated with an already reported clinical event. Laboratory abnormalities associated with a clinical event (e.g., elevated liver enzymes in a subject with jaundice) will be described as a separate AE.

Pre-existing Conditions

In this trial, a pre-existing condition (i.e., a disorder present before the AE reporting period started and noted on the pre-treatment medical history/physical examination form, this will include pregnancy) is not to be reported as an AE unless the condition worsens or episodes increase in frequency during the AE-reporting period.

Procedures

Diagnostic and therapeutic non-invasive and invasive procedures, such as surgery, are not to be reported as AEs. However, the medical condition for which the procedure is performed is to be reported if it meets the definition of an AE. For example, an acute appendicitis that begins during the AE reporting period is to be reported as the AE and the resulting appendectomy noted under Comments.

7.2 Eliciting Adverse Event Information

The investigator is to report all directly observed AEs and all AEs spontaneously reported by the trial subject. In addition, each trial subject will be questioned about AEs at each clinic visit following initiation of treatment

7.3 Adverse Event Reporting Period

The AE reporting period for this trial begins upon randomization, and ends 30 days after receiving the last dose of trial medication. The AE reporting period for neonates begins upon randomization and ends 30 days after the subject has received the last dose of trial medication.

All AEs that occur in trial subjects during the AE reporting period specified in the protocol must be recorded in the CRF, WHETHER OR NOT THE EVENT IS CONSIDERED MEDICATION/PRODUCT RELATED.

IN ADDITION, any known untoward event that occurs subsequent to the AE reporting period that the investigator assesses as possibly related to the investigational medication/product is also to be reported as an AE.

Note that in view of this trial:

- **Injection-site ecchymosis will not be reported as an AE.**
- **The following pregnancy-related symptoms will not be reported as an AE:**
 - **Nausea, vomiting**
 - **Minor vaginal bleeding**
 - **Gradual onset dyspnea**
 - **Fatigue**
 - **Bilateral leg, arm or facial swelling**
 - **Heart burn, weight gain, abdominal distension**

7.4 Recording Instructions

AEs are to be recorded in the case report forms as specified.

If required on the AE case report forms, the investigator will use the adjectives MILD, MODERATE, or SEVERE to describe the maximum intensity of the AE. For purposes of consistency, these intensity grades are defined as follows:

MILD	Does not interfere with subject's usual function
MODERATE	Interferes to some extent with subject's usual function
SEVERE	Interferes significantly with subject's usual function

A distinction is made between the gravity and the intensity of an AE. **Severe** is a measure of intensity; thus, a **severe** reaction is not necessarily a **serious** reaction. For example, a headache may be severe in intensity, but is not to be classified as serious unless it meets one of the criteria for serious events listed in section 7.5. The investigator will also be asked to assess the possible relationship between the AE and the investigational medication as well as any concomitant medications.

7.5 Seriousness (Gravity) – Serious Adverse Events (SAE)

Each AE is to be classified by the investigator as SERIOUS or NONSERIOUS. This classification of the gravity of the event determines the reporting procedures to be followed.

A **SERIOUS adverse event (SAE)** is an AE that meets one or more of the following criteria/outcomes:

- Death
- Life-threatening (i.e., immediate risk of death)
- In-subject hospitalization or prolongation of existing hospitalization
- Persistent or significant disability/incapacity (any sight-threatening event with ophthalmic products is a significant incapacity)

- Permanent impairment of function or permanent damage to a body structure or requires intervention to prevent permanent impairment or damage
- Congenital anomaly/birth defect
- Any other AE that the investigator or company judges to be serious or which is defined as serious by the regulatory agency in the country in which the AE occurred.

Note that in view of this trial:

- **Pregnancy losses where the fetus shows no gross visual evidence of congenital anomalies (when available), will not be reported as a SAE, unless considered to be related to the investigational product.**
- **Admission to hospital for the purpose of term spontaneous vaginal delivery and/or elective caesarean section will not be reported as an SAE.**

Exposure In Utero

If the outcome of the pregnancy meets the criteria for immediate classification as a SAE (i.e., congenital anomaly [including that in an aborted fetus]), the investigator is to follow the procedures for reporting SAEs; i.e., **report the event to the APPLE Multicentre Trial Office in Ottawa Canada by fax and follow up by submission of appropriate case report forms and source documentation.**

Additional information regarding pregnancy outcomes that are classified as SAEs follows:

- "Spontaneous abortion" includes miscarriage and missed abortion, if considered to be related to the investigational product (i.e. congenital anomaly)
- All neonatal deaths that occur within one month of birth are to be reported, without regard to causality, as SAEs. In addition, any infant death after one month that the investigator assesses as possibly related to the *in utero* exposure to the investigational medication are also to be reported.
- The "normality" of an aborted foetus can be assessed by gross visual inspection unless there are preabortion laboratory findings suggestive of a congenital anomaly.

7.6 Reporting of SAEs

If a SAE occurs, the APPLE Multicentre Trial office, Ottawa Canada is to be notified within 24 hours of awareness of the event by the investigator. The SAE report form should be sent to the multicentre coordinator and Principal Investigator as outlined in the APPLE Pilot Resource Manual.

In turn, the Principal Investigator will:

- Review all SAEs and comment via email within 24 hours should clarification be required, and
- If the SAE is related or probably related, and unexpected, the principal investigator will report the SAE to Health Canada using the CIOMS form and notify the REB board of record, Ottawa Health Science Network REB (OHSN-REB) through Clinical Trials Ontario (CTO), as appropriate. Participating sites are responsible for informing their institutions of the event according to their local standard operating procedures.

The initial report is to be followed by submission of more detailed SAE information on the SAE form within 15 working days of the event.

Table 1 REPORTING REQUIREMENTS FOR ADVERSE EVENTS		
Gravity	Reporting Time	Type of Report
SERIOUS (life-threatening or death)	Within 24 hours	Initial report on SAE
	Within 7 days	Final report on SAE
SERIOUS	Within 24 hours	Initial report on SAE
	Within 15 days	Final report on SAE
NON-SERIOUS	Following each of these subject visits: - 20 ± 4 weeks gestational age - Labour & delivery - 6-12 weeks postpartum	Adverse Event CRFs

NOTE: In the rare event that the investigator does not become aware of the occurrence of a SAE immediately (e.g., if a trial subject initially seeks treatment elsewhere), the investigator is to report the event within 24 hours after learning of it and document his/her first awareness of the SAE.

All SAEs are to be reported to and reviewed by the Data Safety Monitoring Board (DSMB) according to the DSMB Terms of Reference.

7.7 Follow-Up of Adverse Event

All AEs are to be followed until they are resolved or the subject's participation in the trial ends (i.e., until a final report is completed for that subject).

In addition, all SAEs and those non serious events assessed by the investigator as possibly related to the investigational medication/product are to continue to be followed even after the subject's participation in the trial is over. Such events are to be followed until they resolve or until the investigator assesses them as "chronic" or "stable." Resolution of such events is to be documented on the appropriate CRF.

8. STATISTICS

8.1 Statistical and Analytical Plans

8.1.1 Analysis of Baseline Characteristics

Descriptive statistics will be used to examine the baseline characteristics of excluded subjects and those in the experimental and standard of care arm. Standard deviations will be reported for all characteristics expressed as continuous variables. Medians and ranges will be presented for discrete data.

8.1.2. Pilot Trial Primary Analysis

The primary analysis will involve a simple estimate of the mean monthly recruitment rate per site along with the 95% confidence interval of the mean.

8.1.3. Pilot Trial Secondary Analyses

Proportions with 95% confidence intervals will be calculated using Wilson's score method for the following secondary analyses: 1) proportion of screened subjects who meet eligibility criteria, 2) proportion of eligible subjects who provide consent, 3) proportion of withdrawals/losses to follow-up among randomized subjects, 4) proportion of sites requiring >18 months to obtain all required approvals/contracts from time of delivery of all study documents, and 5) proportion of crossover between study arms. Reasons for non-consent will be collected and analyzed using qualitative thematic analysis.

8.1.4. Full Trial Analyses

Several secondary outcomes will be collected in the pilot trial, which will be included in the analysis of the full trial if there are no significant changes to the trial design.

Primary analysis: Analysis will be performed by intention to treat. Intention to treat analysis will be supplemented by a sensitivity analysis that excludes subjects who did not complete the allocated treatment plan. The live birth rate will be compared in the experimental and standard of care arm by an unadjusted Fisher's test of proportions, with 95% confidence intervals provided.

Secondary analysis: Analysis will be performed by intention to treat. Intention to treat analysis will be supplemented by a sensitivity analysis that excludes subjects who did not complete the allocated treatment plan. Proportions will be compared between study arms by an unadjusted Fisher's test, with 95% confidence intervals reported. The following outcomes will be analyzed: Pregnancy loss by central laboratory testing, pregnancy loss by gestational age (<10 weeks, 10-19 weeks, ≥20 weeks gestation), pre-eclampsia, early onset pre-eclampsia, severe pre-eclampsia, SGA <10th percentile, SGA <5th percentile, SGA <3rd percentile, placental abruption requiring delivery, pre-term delivery <37 weeks gestation, symptomatic VTE, symptomatic ATE, major bleeding, clinically relevant non-major bleeding, non-major non-CRNM, major peripartum bleeding, minor peripartum bleeding, HIT, symptomatic fracture, allergic reaction, and all-cause mortality.

8.2 Determination of Sample Size

The sample size of the pilot trial is 24 participants, based on a recruitment rate of 1 participant per 2 months per site. This sample size was derived based on the full trial sample size, which factored in that these 2 sites are highly motivated and will have higher recruitment rates compared to other potential sites.

The full trial sample size is 828 participants, which is based on a 60% absolute event rate in the ASA arm, minimal clinically important difference (MCID) of 10%, 80% power at alpha 0.05, and a drop-out rate of 10%. The MCID was determined based on a survey of 22 physicians (20% response rate) among CanVECTOR Network and Thrombosis Canada members.

With involvement from 40 centers over 5 years, this sample size calculation of 828 equates to 4 participants per year per center, however, we acknowledge that the 2

pilot sites are highly motivated and will have higher recruitment rates on average, therefore we have set a minimum recruitment rate of 1 patient per 2 months per site.

9. QUALITY ASSURANCE (QA)

9.1. Quality Assurance

Quality assurance at trial sites will be supported through training and monitoring. At each site this will consist of an initiation visit or teleconference, ongoing training (as needed), central and on-site monitoring, and a close-out visit or teleconference. Details about the process and content of the initiation and close-out visits are included in the Resource Manual.

9.2 Monitoring

Study monitoring is a sponsor responsibility that ensures that the rights and well-being of human subjects are protected, the reported trial data are accurate, complete, and verifiable from source documents, and that the conduct of the trial complies with the protocol, GCP, and regulatory requirements. Monitoring for the trial will be performed by the sponsor, according to the detailed monitoring plan for the trial.

10. ETHICS

10.1 Ethical Conduct of the Trial

The trial will be performed in accordance with the recommendations guiding physicians in biomedical research involving human subjects adopted by the 18th World Medical Assembly (Helsinki, Finland, 1964 and later revisions), the Tri-Council Policy Statement and the ICH GCP Guidelines.

10.2 Research Ethics Board (REB)

REB approval will be obtained and maintained annually with OHSN-REB, the REB board of record, through CTO.

10.3 Subject Information and Consent

The investigator, or his designee, will inform each subject (or the subject's acceptable representative) prior to inclusion in the trial, full and adequate verbal and written information regarding the objective and procedures of the trial and the possible risks involved. The subjects must be informed about their right to withdraw from the trial at any time. Written subject information will be given to each subject before enrolment. Furthermore, it is the responsibility of the investigator or his designee to obtain signed informed consent from all subjects prior to inclusion in the trial.

11. DATA SAFETY MONITORING BOARD

11.1 Data Safety Monitoring Board (DSMB)

A DSMB will complete a safety analysis after 16 patients are recruited with a pre-defined stopping rule. The DSMB Terms of Reference outline the DSMB operating procedures.

11.2. Interim Stopping Rule

The pilot study will be halted early if 0 out of 8 subjects in either arm have a live birth. The upper bound of the confidence interval is 32.4%, which would be lower than expected. If only 1/8 patients had a live birth, while the point estimate is 12.5%, the upper bound of the 95th confidence interval is 47.1%, which is still in the realm of expected results.

12. DATA HANDLING AND RECORD KEEPING

12.1 Personal Health Information

Medical records will be reviewed in compliance with applicable provincial privacy laws as well as local hospital privacy policies. All personal health information will be treated in a confidential manner with respect to its collection, use, and disclosure. Participant names or potentially identifying personal health information will not leave the institution. Any source documents provided to the sponsor for monitoring purposes must first be de-identified. The consent form will describe to the participant who will have access to their information and for what purposes (e.g., Health Canada, Research Ethics Board (REB), research sponsors and personnel monitoring/auditing the research on their behalf). A master list that links participant identities to their unique participant numbers will be maintained at all study sites and stored separately from all other study records.

12.2 Case Report Forms

A Case Report Form (CRF) is required and will be completed for each randomized subject. The completed original CRFs are the sole property of The Ottawa Hospital and are not to be made available in any form to third parties, except for authorized representatives of appropriate Regulatory Authorities.

12.3 Record Retention

To enable evaluations and/or audits, the investigator will keep records according to section 8.0 in ICH/GCP, including the identity of all participating subjects (sufficient information to link records, e.g., CRFs and hospital records), all original signed Informed Consent Forms and source documents, copies of all CRFs and detailed records of drug disposition. To comply with Canadian regulations, the records are to be retained for 25 years.

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