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Complete List of Authors:	O'Gorman, Neil; King\'s College Hospital, Fetal Medicine Research Institute Wright, David ; University of Exeter, Institute of Health Research Rolnik, Daniel; King\'s College Hospital, Fetal Medicine Research Institute Nicolaides, Kypros; King\'s College Hospital, Fetal Medicine Research Institute Poon, Liona; King\'s College Hospital, Fetal Medicine Research Institute
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ASPRE: Combined multi-marker screening and randomised patient treatment with ASpirin for evidence-based PREeclampsia prevention

Neil O'Gorman[1], David Wright[2], Daniel L. Rolnik[1], Kypros H. Nicolaides[1]*, Liona C. Poon[1]*.

*Senior authors

Correspondence
Liona C. Poon
Harris Birthright Research Centre for Fetal Medicine
Division of Women's Health
King's College London,
Denmark Hill, London SE5 9RS
Telephone +44 20 3299 8256
Fax +44 20 7733 9543
Mail: chiu_yee_liona.poon@kcl.ac.uk

Author affiliations
1. Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, London, UK.
2. Institute of Health Research, University of Exeter, Exeter, UK.

Contributors
LCP and KHN conceived and designed the study. LCP, KHN, NOG, and DLR drafted the original grant proposal and trial protocol. DW provided methodological and statistical expertise. LCP and KHN provide expertise in the pregnancy clinical outcomes. LCP, KHN, NOG, DLR and the Clinical Project Manager drafted the original protocol. LCP and NOG drafted the manuscript. LCP, NOG, DLR, with the support of the Trial Manager and the Clinical Project Manager, have responsibilities for day-to-day running of the trial including participant recruitment, data collection and liaising with other sites. All authors critically reviewed and approved the final version of the manuscript.

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Competing interests None declared

Disclaimer The views expressed in this publication are those of the author(s) and not necessarily those of the FMF, European Union FP7, healthcare systems or competent authorities.

Ethics approval This study will be conducted in accordance with the principles of Good Clinical Practice. This protocol was submitted to the National Research Ethics Committee and a favourable ethical opinion was granted. The reference number is 13/LO/1479 Subsequent approval by individual ethical committee and competent authority was granted.

Trial Sponsor University College London, Comprehensive Clinical Trials Unit. The trial sponsor will act as custodian of the data.

Susan Tebbs
Deputy Director
UCL CCTU
Tel 020 3549 5017
Mobile 07788 396029

Provenance and peer review Not commissioned; peer reviewed for ethical and funding approval prior to submission.

Strengths and Limitations

- This is the largest multi-centre, double-blinded, randomised placebo-controlled trial to examine the effect of aspirin in women who are high risk of developing preeclampsia.
- The screening will occur in the first-trimester as to allow for the maximum benefit of aspirin.
- 150 mg of aspirin will be used to reduce the incidence of aspirin resistance and maximise the effect.
- Follow-up of the offspring is limited to the early postnatal phase.

Abstract

Introduction: Preeclampsia (PE) affects 2-3% of all pregnancies and is a major cause of maternal and perinatal morbidity and mortality. Prophylactic use of low-dose aspirin in women at risk for PE may substantially reduce the prevalence of the disease. Effective screening for PE requiring delivery before 37 weeks' (preterm-PE) can be provided by a combination of maternal factors, uterine artery Doppler, mean arterial pressure, maternal serum pregnancy-associated plasma protein-A and placental growth factor at 11-13 weeks' gestation, with a detection rate of 75% at a false-positive rate of 10%. We present a protocol (version 6, date 25.01.2016) for the ASPRE trial, which is a double-blinded, placebo-controlled, randomised controlled trial (RCT) that utilises an effective PE screening programme to determine whether low-dose aspirin given to women from 11-13 weeks' gestation will reduce the incidence of preterm-PE.

Methods and analysis: All eligible women attending for their first-trimester scan will be invited to participate in the screening study for preterm-PE. Those found to be at high-risk of developing preterm-PE will be invited to participate in the RCT. Further scans will be conducted for assessment of fetal growth and biomarkers. Pregnancy and neonatal outcomes will be collected and analysed. The first enrolment for the pilot study was in April 2014. As of December, 11,000, women have been screened and 900 recruited to the RCT.

Ethics and dissemination: The study will be conducted in accordance with the principles of Good Clinical Practice. A favourable ethical opinion was obtained from London-Fulham Research Ethics Committee, reference number 13/LO/1479. Subsequent approval by individual ethical committee and competent authority was granted. Results will be published in peer-reviewed journals and disseminated at international conferences.

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Background

Preeclampsia (PE) is an important cause of maternal and perinatal mortality and morbidity. There is extensive evidence that the risk of adverse outcome in relation to PE is much higher when the disease is severe and of early onset requiring delivery before 37 weeks' gestation (preterm-PE), than at term.[1-4] A major challenge in modern obstetrics is early identification of pregnancies at high-risk of preterm-PE and undertaking the necessary measures to improve placentation and reduce the prevalence of the disease.

Prediction of preterm preeclampsia

Extensive research in the last 20 years, mainly as a consequence of the shift in screening for aneuploidies from the second to the first-trimester of pregnancy, has identified a series of early biophysical and biochemical markers of impaired placentation.[5] A combination of maternal demographic characteristics, including medical and obstetric history, uterine artery pulsatility index (PI), mean arterial pressure (MAP) and maternal serum pregnancy associated plasma protein-A (PAPP-A) and placental growth factor (PlGF) at 11-13 weeks' gestation can identify a high proportion of pregnancies at high-risk for PE.[6] A recent study involving 39,000 singleton pregnancies examined at 11-13 weeks' gestation has further refined the prediction algorithm for PE. Using this algorithm the estimated detection rate of preterm-PE was 75%, at a false positive rate of 10%.[6]

Prevention of preterm preeclampsia

The prophylactic use of low-dose aspirin for prevention of PE has been an important research question in obstetrics for the last three decades. In 1979, Crandon and Isherwood observed that nulliparous women who had taken aspirin regularly during pregnancy were less likely to have PE than those who did not.[7] A meta-analysis reported that the administration of low-dose aspirin in high-risk pregnancies is associated with a decrease in the rate of PE by approximately 10%.[8]

Initiation of low-dose aspirin in early pregnancy

In most studies that evaluated aspirin for the prevention of PE, the initiation of treatment was at or after 16 weeks' gestation. Examination of a small number of randomised trials of low-dose aspirin in women at high-risk for PE suggests that the effectiveness of therapy is related to the gestational age at the initiation of treatment. A meta-analysis by Bujold *et al.* reported that low-dose aspirin started at 16 weeks' or earlier was associated with a significant reduction in the relative risk (RR) for PE (0.47, 95% confidence interval [CI] 0.34-0.65) and fetal growth restriction (FGR) (0.44, 95% CI 0.30-0.65).[9] In contrast, aspirin started after 16 weeks did not have a significant benefit (PE: RR 0.81, 95% CI 0.63-1.03; FGR: RR 0.98, 95% CI 0.87-1.10). More detailed analyses of these data on PE demonstrated that low-dose aspirin started at or before 16 weeks' gestation was particularly effective in preventing preterm-PE rather than term-PE (RR: 0.11, 95% CI 0.04-0.33 vs. RR: 0.98, 95% CI 0.42-2.33).[10]

The small number and small size of individual trials preclude definitive conclusions to be drawn regarding the effectiveness of aspirin starting before 16 weeks' and the results need to be examined in a prospective major randomised trial.

Aspirin resistance

Low-dose aspirin is defined as less than 300 mg per day. There is evidence that approximately 30%, 10% and 5% of pregnant women are "aspirin resistant" with dosage of 81 mg, 121 mg, and 162 mg, respectively.[11] Furthermore, a retrospective cohort study reported that women who were identified by the PFA-100 test as being resistant to 81 mg of aspirin were less likely to develop severe PE when the dose of aspirin was increased from 81 to 162 mg, compared to those who continued with 81 mg.[12] Consequently, a trial investigating the effectiveness of low-dose aspirin in the prevention of preterm-PE should use a dose closer to 160 mg than 80 mg.

Safety of low-dose aspirin

The relative safety of first-trimester use of low-dose aspirin has been demonstrated in large cohort and case-control studies, which reported that the drug is not associated with increase in risk of congenital heart defects or other structural or developmental anomalies.[13-16]

Randomised studies reported that approximately 10% of women receiving low-dose aspirin complained of gastro-intestinal symptoms; however there was no evidence of increase in any type of maternal bleeding.[17-19] Similarly, the best evidence suggests that low-dose aspirin started before 16 weeks' gestation does not increase the risk of placental abruption (RR: 0.62, 95% CI 0.08–5.03).[9] No additional adverse effects related to epidural anaesthesia have been reported in women taking low-dose aspirin compared to those taking placebo.[20]

Prospective and case-control studies did not find an association between daily consumption of 60-150 mg of aspirin during the third-trimester and antenatal closure of the ductus arteriosus.[21-23] A meta-analysis including more than 26,000 women randomised to low-dose (80-150 mg) aspirin or placebo/no treatment during pregnancy demonstrated that the use of aspirin was not associated with an increase in intra-ventricular haemorrhage or other neonatal bleeding.[24] On the basis of currently available evidence it would be reasonable to continue with low-dose aspirin well into the third-trimester of pregnancy.

Hypothesis

We hypothesise that prophylactic low-dose aspirin administered from first-trimester of pregnancy in women at increased risk for PE will reduce the incidence and severity of the disease.

Aim

To examine if the prophylactic use of low-dose aspirin administered from the first-trimester of pregnancy in women at increased risk for PE can reduce the incidence and severity of the disease.

Objectives

Primary objective

To determine the efficacy of low-dose aspirin (150 mg daily), given to high-risk women from 11-14 weeks' gestation until 36 weeks', in reducing the incidence of preterm-PE, requiring delivery before 37 weeks.

Secondary objectives

- To determine the effect of low-dose aspirin on adverse outcome of pregnancy at <37 weeks.
  - PE requiring delivery at <37 weeks'
  - SGA (<5th percentile) requiring delivery at <37 weeks'
  - Miscarriage or stillbirth at <37 weeks'
  - Placental abruption (clinically or on placental examination) at <37 weeks'
  - Composite of any of the above
- To determine the effect of low-dose aspirin on adverse outcome of pregnancy at <34 weeks.
  - PE requiring delivery at <34 weeks'
  - SGA (<5th percentile) requiring delivery at <34 weeks'
  - Miscarriage or stillbirth at <34 weeks'
  - Placental abruption (clinically or on placental examination) at <34 weeks'
  - Composite of any of the above
- To determine the effect of low-dose aspirin on adverse outcome of pregnancy at ≥37 weeks.
  - PE requiring delivery at ≥37 weeks'
  - SGA (<5th percentile) requiring delivery at ≥37 weeks'
  - Miscarriage or stillbirth at ≥37 weeks'
  - Placental abruption (clinically or on placental examination) at ≥37 weeks'
  - Composite of any of the above
- To determine the effect of low-dose aspirin on neonatal mortality and morbidity.
  - Neonatal intensive care unit admission
  - Intraventricular haemorrhage (IVH) grade II or above - Defined as bleeding into the ventricles
    - *Grade II (moderate)* – IVH occupies <50% of the lateral ventricle volume
    - *Grade III (severe)* – IVH occupies >50% of the lateral ventricle volume
    - *Grade IV (severe)* – Haemorrhagic infarction in periventricular white matter ipsilateral to a large IVH
  - Ventilation - Defined as need of positive pressure (continuous positive airway pressure (CPAP) or nasal continuous positive airway pressure (NCPAP)) or intubation
  - Neonatal sepsis - Confirmed bacteraemia in cultures
  - Anaemia – Defined as low haemoglobin and / or haematocrit requiring blood transfusion
  - Respiratory distress syndrome - Defined as need of surfactant and ventilation as a result of prematurity
  - Necrotising enterocolitis (NEC) requiring surgical intervention
    NEC is defined by a combination of clinical, radiological and laboratory features:
    - *Systemic signs* - apnoea, bradycardia, temperature instability, hypotension.
    - *Intestinal signs* - abdominal distension, gastric residuals, bloody stools, absent bowel sounds, abdominal tenderness, peritonitis.
    - *Radiological signs* - pneumatosis intestinalis or portal venous air, pneumoperitoneaum.
    - *Laboratory changes* - metabolic and or respiratory acidosis, thrombocytopaenia, DIC.
  - Composite of any of the above
- To determine the effect of low-dose aspirin on the incidence of neonatal birthweight below the 3rd, 5th and 10th centile.

- Birthweight will be recorded in the participants' medical notes and birthweight percentile for gestational age at delivery is calculated using a normal range derived from our population.[25]

- To determine the effect of low-dose aspirin on the incidence of stillbirth or neonatal death.
  - Due to any cause
  - Ascribed to PE or FGR
  - In association with maternal or neonatal bleeding
- To determine the effect of low-dose aspirin on the incidence of spontaneous preterm delivery at <34 weeks and <37 weeks.
  - Spontaneous delivery at <34 weeks (early preterm) and at <37 weeks (total preterm) includes those with spontaneous onset of labour and those with preterm pre-labour rupture of membranes.

Centres

Thirteen centres participating in the trial. There are six centres in the United Kingdom, three in Spain, and one in each of Milan, Brussels, Greece and Israel.

Design

There are three components to the study: an internal pilot study, a screening quality study and a screening study followed by a double-blinded randomised placebo-controlled trial.

Internal pilot study

The main study has been preceded by a two-month pilot study, undertaken at King's College Hospital. 1,106 participants have been consented into the screening study and 56 participants to the RCT. This pilot study has been used to assess the feasibility of recruitment to both the screening study and RCT and the ability of the centre to ensure successful compliance. A review by the ASPRE Independent Data Monitoring Committee (IDMC) and Trial Steering Committee (TSC) of the internal pilot study has demonstrated the study has been successful with respect to recruitment to both the screening study and RCT; however it has also highlighted the complexity of the main ASPRE trial and confirmed the need for enhanced quality systems to be in place in advance of starting the main ASPRE trial in order to ensure the quality of pivotal data. □

Screening Quality Study

A screening quality study, with a minimum recruitment period of one to three months (dependent on sites' performance) at each site, has been introduced to precede the main ASPRE trial. The aim of this study is to establish systems that will monitor quality of the measurement of uterine artery PI, MAP, PAPP-A and PlGF in a more detailed, formalised manner at sites and use these systems to assess quality, identify areas for improvement and, where required, implement strategies to improve quality e.g. re-training. This is based on the DQASS system that has been successful for improving the quality of the ultrasound and biochemical measurements in the NHS fetal anomaly screening programme.

Recruitment rates have also been monitored. Furthermore, an assessment of data quality was made by the trial team at UCL CCTU and any site-specific operational issues, which could not have been foreseen by the site assessment process, were identified, and addressed in advance of starting the main ASPRE trial.

Screening Study and Randomised Control Trial

Following receipt of the result of the screening study, eligible high-risk women will be invited to take part in the RCT by designated the trial teams. It is anticipated that 10% of the population will screen positive for preterm-PE and be invited to participate in the trial (Figure 1).

Inclusion and exclusion criteria

Screening phase inclusion criteria

- Age ≥ 18 years;
- Singleton pregnancy;
- Live fetus at 11-13 weeks' of gestation;
- English, Italian, Spanish, French, Dutch or Greek speaking (otherwise interpreters will be used);
- Informed and written consent.

Screening phase exclusion criteria

- Multiple pregnancy;
- Pregnancies complicated by major fetal abnormality identified at the 11-13 weeks assessment;
- Women who are unconscious or severely ill, those with learning difficulties, or serious mental illness;
- Age < 18 years.

Randomisation inclusion criteria following screening

- Screening phase inclusion criteria fulfilment;
- High-risk for preterm-PE at 11-13 weeks' by the algorithm combining maternal history and characteristics, biophysical findings (MAP and uterine artery PI) and biochemical factors (PAPP-A and PlGF).

Randomisation exclusion criteria following screening

- Women taking low-dose aspirin regularly;
- Bleeding disorders such as Von Willebrand's disease;
- Peptic ulceration;
- Hypersensitivity to aspirin or already on long term non-steroidal anti-inflammatory medication;
- Concurrent participation in another drug trial or at any time within the previous 28 days;
- Any other reason the clinical investigators think will prevent the potential participant from complying with the trial protocol.

Methods

We will recruit women attending for their routine first scan in pregnancy at 11-13 weeks' gestation in the UK, Spain, Belgium, Italy, Greece and Israel. All eligible women attending for their routine first scan in pregnancy at 11-13 weeks' gestation are invited to take part. For the screening quality study and the screening study the PIS will be sent with the appointment letter to all potential participants.

In women who agree to participate in the screening quality study, after obtaining informed consent, we measure the maternal MAP by automated devices,[26] use transabdominal colour Doppler ultrasound to visualise the left and right uterine artery and measure the PI in each vessel and calculate the mean PI.[27] Maternal serum PlGF is measured in the same blood sample taken for the measurement of PAPP-A, using automated machines that provide reproducible results (DELFIA Xpress system, PerkinElmer Life and Analytical Sciences, Waltham, USA) as part of the routine screening for Down's syndrome. Participants enrolled in the screening quality study will not be informed of their risk of developing PE and will be managed according to routine standard of care at the site they attend. The Principal Investigators at each site are doctors who received their training by Professor Nicolaides and follow the Fetal Medicine Foundation (FMF) guidelines on how to undertake the appropriate measurements.

In women who agree to participate in the screening study of the main trial, after obtaining informed consent, we measure maternal MAP, uterine artery PI, PAPP-A and PlGF as described above. Following screening for preterm-PE, high-risk women will be invited to take part in the RCT by designated members of the trial teams. Women eligible to participate in this trial will receive written information on the test drug and provide informed consent. When randomised, participants will be assigned a randomisation code. The randomisation codes will determine who receives placebo or aspirin 150 mg. The IMP supplier, Mawdsley Brooks and Co. will keep and store the randomisation code list. All participants, the PI and clinical trial pharmacy will remain blind to trial drug allocation.

Data Collection

Participant data for this study will be entered into an electronic case report form (CRF). For participants in the RCT that will be printed and signed by the enrolling researcher.

Randomisation

Randomisation will be performed using a web-based system Sealed Envelope. The website randomly assigns participants to a randomisation code which correspond to treatment packs with the same code at a given site. Each treatment pack will only be identified by a randomisation code. The treatment allocation will only be revealed to the researchers after completion of the study or where clinically essential.

Concealment of allocation

Mawdsley Brooks and Co. will provide labelling (for all cartons and blister sheets) ensuring complete blinding of the investigational medicinal product (IMP) to all investigators and participants in the study. That includes the PI, participating research doctors, pharmacists at the local clinical trial pharmacy, project managers and others involved in the trial. They are all blinded to the IMP allocation. Matching placebo tablets will be identical to the intervention (aspirin) in such parameters as size, thickness, physical properties and appearance. A film coating will be applied to the placebo tablets for aesthetic and taste reasons.

Mawdsley Brooks and Co. will keep the randomisation code list confidential to maintain the blind, however the randomisation code list will be transferred to Sealed Envelope to enable to online randomisation and unblinding service to be established.

Intervention

Participants will take one tablet per night of either aspirin 150 mg or matched placebo. Participants will be asked to stop taking tablets at 36 weeks' gestation or, in the event of early delivery, at the onset of labour (maximum duration of 25 weeks). The aspirin tablets will be film-coated, to be taken orally once per night from enrolment until 36 weeks' gestation.

Study assessment

The study procedure by visit has been outlined in table 1.

Laboratory Tests

At the time of the 11-13 weeks scan, 20 mL of maternal blood will be taken for the measurement of PAPP-A and PlGF using automated machines that provide reproducible results (DELFIA Xpress system, PerkinElmer Life and Analytical Sciences, Waltham, USA). The remaining serum and plasma will be stored at -80^{o}C for future studies of potential biochemical markers for adverse pregnancy outcomes.

Participant compliance

Participants will be asked to bring their trial medication to each clinical visit; IMP compliance will be assessed by trial teams by counting remaining tablets at each follow up visit and asking about compliance at telephone follow up. Compliance with other aspects of the trial protocol will also be assessed. Participants will be encouraged to report any concerns or side effects in a diary for review at each trial visit.

Outcomes

Primary outcome

- Incidence of preterm-PE (delivery at <37 weeks)

PE will be defined as per the International Society for the Study of Hypertension in Pregnancy.[29] The systolic blood pressure should be 140 mm Hg or more and/or the diastolic blood pressure should be 90 mmHg or more on at least two occasions four hours apart developing after 20 weeks of gestation in previously normotensive women (blood pressure less than 140/90 mmHg) and there should be proteinuria of 300mg or more in 24 hours or urinary protein creatinine ratio of 30 mg/mmol or more or two readings of at least ++ on dipstick analysis of midstream or catheter urine specimens if no 24-hour collection is available. The efficacy will be assessed by the development of PE at any gestation after 20 weeks of pregnancy as defined above.

Secondary outcomes

- As defined above in the secondary objectives section.

Collection of pregnancy and neonatal outcomes

Data on pregnancy and neonatal outcomes will be collected from the hospital maternity records or their general medical practitioners. The obstetric records of the randomised women with pre-existing or pregnancy-associated hypertension will be examined to determine if the condition was chronic hypertension, PE or gestational hypertension. In the event neonates are admitted to Special Care Baby Unit (SCBU), additional neonatal outcomes will be collected from the discharge summary of SCBU.

Side effects and adverse events reporting

Adverse event (AE) and reaction (AR) data are not being collected for participants of the screening quality study, or the screen-negative participants in main ASPRE RCT, as they are non-CTIMPs which do not expose participants to any additional risk over and above that of routine clinical care.

Safety evaluations will be conducted at each of the RCT participants' follow-up visits. Adverse events include any unwanted side effects, sensitivity reactions, abnormal laboratory results, injury or inter-current illnesses, and may be expected or unexpected. The period for AE reporting will be from the time of first dose until 30 days post final IMP administration. The participants will be followed up by a telephone interview 30 days after the last dose of IMP. These AEs will be recorded on the electronic CRF and do not need to be reported to the Sponsor. The participants are instructed to contact a member of the trial team if there are any concerns regarding their medication.

Serious adverse events/reactions (SAE/SAR) occurring in the mother or baby from the time a participant is randomised until 30 days after stopping taking the IMP or until 30 days after delivery or until 30 days after the estimated due date, respectively, whichever is later, will be reported to the Sponsor using the trial documentation. The standard definition of a SAE will be used.[30]

For the purposes of this study the following events are included as protocol defined exceptions to SAE reporting should only be reported to the Sponsor as an SAE/SAR if the investigator believes the event is a result of the ASPRE intervention: hospitalisation for maternal or fetal observation, including minor bleeding episodes; preterm delivery (spontaneous, for maternal or fetal indication); miscarriage; stillbirth or neonatal death; admission of baby to Neonatal Intensive Care Unit; termination for fetal or maternal indication. If the event is deemed to be part of the routine progress of the pregnancy concerned, these events should be reported to the Sponsor as a protocol defined exception to SAE reporting, within the respective reporting timelines.

Statistical analysis plan including sample size and power calculation

The sample size calculation is based on a 76% detection rate of the first-trimester combined screening for preterm-PE at a screen positive rate of 10%. With the aim to achieve a significant 50% reduction in the prevalence of preterm-PE from 7.6% in the placebo group to 3.8% in the aspirin group, with a power of 90%, and 5% significance level, it is necessary to randomise 1,600 high-risk pregnancies. If we allow for 10% loss to follow up, it will be necessary to randomise a total of 1,760 high-risk pregnancies, 880 women in each of the aspirin and placebo arms. On the assumption that 60% of high-risk pregnancies will agree to randomisation we need to identify 2,933 high-risk pregnancies (that will constitute 10% of the screened population). We will therefore have to recruit a total of 29,330 pregnancies to the screening study.

Type of analysis and statistical tests

The primary analysis will comprise an intention-to-treat comparison of the two groups with respect to the proportion of high-risk pregnancies that develop preterm-PE at the two tailed 5% level. 95% confidence intervals will be produced for the proportions developing preterm PE in each of the two groups and for the difference (active – placebo).

Planned secondary analysis of the primary outcome will include a survival analysis of the time to delivery with PE treating births for other causes as censoring. Pre-specified baseline variables considered to be predictive will be included as appropriate. Their interactions with the treatment effect will be investigated. Gestational age at randomisation and its interaction with treatment will also be investigated. This analysis of treatment interactions will be considered as exploratory.

Descriptive statistics

A full set of descriptive statistics for all variables, overall and by treatment group, will be produced. Graphical displays will be produced as appropriate.

Secondary Analysis

Secondary outcomes will be compared across treatment groups using appropriate tests. P values and 99% confidence intervals will be produced for treatment effects. No corrections will be made for multiplicity.

Safety

The incidence rates of adverse events and serious adverse events and their relationship to trial drugs will be summarized by treatment group. The proportion of women discontinuing treatment will be summarized by reason and by treatment group.

Committee Oversights

The IDMC is independent from the trial and is responsible for monitoring the progress of the trial including: recruitment, protocol adherence, SAEs and side effects of treatment as well as the difference between the trial treatments on the primary outcome measures. They are the only oversight body that has access to unblinded accumulating comparative data. The IDMC is responsible for safeguarding the interests of trial participants, monitoring the accumulating data and making recommendations to the TSC on whether the trial should continue as planned.

The TSC is the independent group responsible for oversight of the trial in order to safeguard the interests of trial participants. The TSC provides advice to the Chief Investigator, Co-Chief Investigator, UCL CCTU, the funder and sponsor on all aspects of the trial through its independent Chair.

Discussion

The traditional approach to screening for PE is to identify risk factors from maternal demographic characteristics and medical history, but such an approach can identify only 35% of total PE and about 40% of preterm-PE at false-positive rate of about 10%.[31,32]

In a proposed new approach to antenatal care, the potential value of an integrated clinic at 11-13 weeks' gestation in which maternal characteristics and history are combined with the

results of a series of biophysical and biochemical markers to assess the risk for a wide range of pregnancy complications has been extensively documented.[33] Effective screening for preterm-PE can be achieved in this clinic with a detection rate of about 76% at a false-positive rate of 10%.[6] There is a suggestion that the prevalence of PE can be halved by prescribing pregnant women low-dose aspirin before 16 weeks' gestation[9] and by using an enhanced screening approach utilising maternal demographics and history with both biochemical and biophysical markers, these women can be identified effectively and entered into a double-blinded randomised placebo-controlled trial to assess whether low-dose aspirin can truly reduce the prevalence of preterm-PE when given in the first-trimester of pregnancy.

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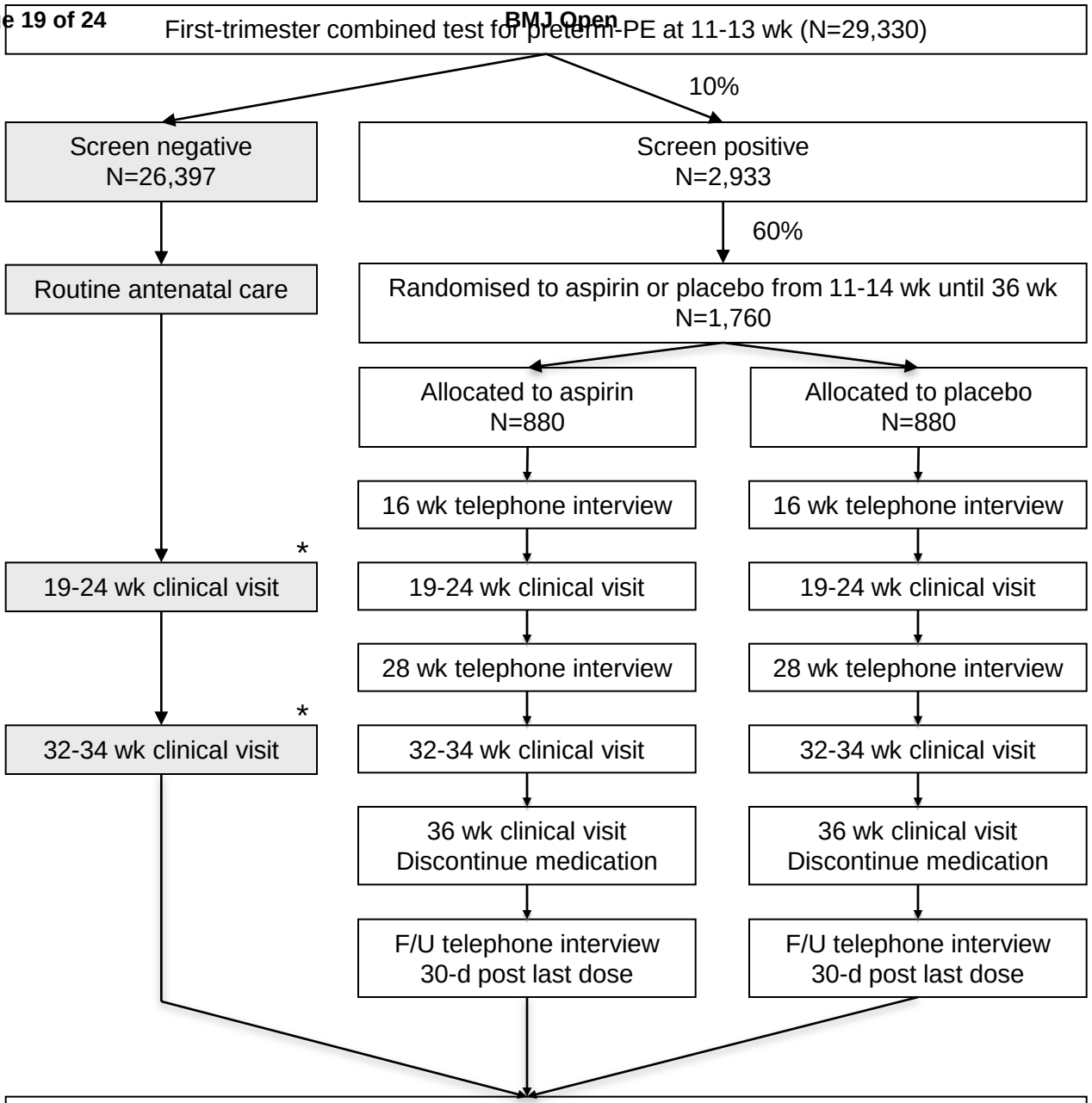
Figure legend

Figure 1. Flow chart of participants in the Screening Study and the Randomised Control Trial. *Clinical visits at 19-24 weeks and 30-37 weeks will only be performed on screen-negative participants at sites where a scan is performed by the fetal medicine unit as part of the routine clinical care pathway at either of these times.

Table 1: Summary of the study visits

	Screening Visit	Randomisation Visit	First telephone interview	First follow up visit	Second telephone interview	Second follow up visit	Third follow up visit	Third telephone interview
Gestation (weeks)	11-13	11-14	16	19-24	28	32-34	36	30 days after the last dose of IMP
Patient information and characteristics	√							
Informed consent	√	√						
Measurement of weight and height	√			√		√	√	
Measurement of MAP	√			√		√	√	
Fetal ultrasound scan	√			√		√	√	
Measurement of uterine artery PI	√			√		√	√	
Measurement of PAPP-A and PlGF	√			√		√		
Check concomitant medications		√	√	√	√	√	√	
IMP dispensing		√		√				
Ensure compliance			√	√	√	√	√	
Check side effects/adverse events and review of diary card			√	√	√	√	√	√
Discontinue IMP							√	



Extraction of pregnancy outcome (anticipating 10% lost to follow up)

Primary outcome

Incidence of preterm-PE

Secondary outcomes

Incidence of early-PE and total PE (at any gestation); birthweight below the 3rd, 5th and 10th centile; stillbirth or neonatal death due to any cause; stillbirth or neonatal death ascribed to PE or FGR; Stillbirth or neonatal death in association with maternal or neonatal bleeding; rate of neonatal intensive care unit admission; composite measure of neonatal mortality and morbidity; placental abruption (clinically or on placental examination); spontaneous preterm delivery <34 weeks and <37 weeks.

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
	2b	All items from the World Health Organization Trial Registration Data Set	1,2,3,4,7,8,9,10,11
Protocol version	3	Date and version identifier	3
Funding	4	Sources and types of financial, material, and other support	1
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1
	5b	Name and contact information for the trial sponsor	2
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	1
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	12

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4,5
	6b	Explanation for choice of comparators	9
Objectives	7	Specific objectives or hypotheses	5,6,7
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	7
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	7
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	8,9
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	10
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	11
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	7,8
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	8,10
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	10,11
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	10,18 & figure 1

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	11
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	8
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	9
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	9
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	9
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	9
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	9
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	10,11
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	10,11

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	9
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	11,12
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	12
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	Not applicable
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	12
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	12
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	11
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	6,14
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	3
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	Not applicable

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	8
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	Not applicable
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	9
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	1
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	2
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	Not applicable
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	3
	31b	Authorship eligibility guidelines and any intended use of professional writers	1
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	Not applicable
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	8
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	10

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

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A study protocol for the Randomised Control Trial: Combined multi-marker screening and randomised patient treatment with ASpirin for evidence-based PREeclampsia prevention (ASPRE)

Neil O'Gorman[1], David Wright[2], Daniel L. Rolnik[1], Kypros H. Nicolaides[1]*, Liona C. Poon[1]*.

*Senior authors

Correspondence
Liona C. Poon
Harris Birthright Research Centre for Fetal Medicine
Division of Women's Health
King's College London,
Denmark Hill, London SE5 9RS
Telephone +44 20 3299 8256
Fax +44 20 7733 9543
Mail: chiu_yee_liona.poon@kcl.ac.uk

Author affiliations
1. Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, London, UK.
2. Institute of Health Research, University of Exeter, Exeter, UK.

Contributors
LCP and KHN conceived and designed the study. LCP, KHN, NOG, and DLR drafted the original grant proposal and trial protocol. DW provided methodological and statistical expertise. LCP and KHN provide expertise in the pregnancy clinical outcomes. LCP, KHN, NOG, DLR and the Clinical Project Manager drafted the original protocol. LCP and NOG drafted the manuscript. LCP, NOG, DLR, with the support of the Trial Manager and the Clinical Project Manager, have responsibilities for day-to-day running of the trial including participant recruitment, data collection and liaising with other sites. All authors critically reviewed and approved the final version of the manuscript.

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Disclaimer The views expressed in this publication are those of the author(s) and not necessarily those of the FMF, European Union FP7, healthcare systems or competent authorities.

Trial Sponsor University College London, Comprehensive Clinical Trials Unit. The trial sponsor will act as custodian of the data and will have disclosure of contractual agreements.

Susan Tebbs
Deputy Director

UCL CCTU
Tel 020 3549 5017
Mobile 07788 396029

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Strengths and Limitations

- This is the largest multi-centre, double-blinded, randomised placebo-controlled trial to examine the effect of aspirin in women who are high risk of developing preeclampsia.
- The screening will occur in the first-trimester as to allow for the maximum benefit of aspirin.
- 150 mg of aspirin will be used to reduce the incidence of aspirin resistance and maximise the effect.
- Follow-up of the offspring is limited to the early postnatal phase.

Abstract

Introduction: Preeclampsia (PE) affects 2-3% of all pregnancies and is a major cause of maternal and perinatal morbidity and mortality. Prophylactic use of low-dose aspirin in women at risk for PE may substantially reduce the prevalence of the disease. Effective screening for PE requiring delivery before 37 weeks' (preterm-PE) can be provided by a combination of maternal factors, uterine artery Doppler, mean arterial pressure, maternal serum pregnancy-associated plasma protein-A and placental growth factor at 11-13 weeks' gestation, with a detection rate of 75% at a false-positive rate of 10%. We present a protocol (version 6, date 25.01.2016) for the ASPRE trial, which is a double-blinded, placebo-controlled, randomised controlled trial (RCT) that utilises an effective PE screening programme to determine whether low-dose aspirin given to women from 11-13 weeks' gestation will reduce the incidence of preterm-PE.

Methods and analysis: All eligible women attending for their first-trimester scan will be invited to participate in the screening study for preterm-PE. Those found to be at high-risk of developing preterm-PE will be invited to participate in the RCT. Further scans will be conducted for assessment of fetal growth and biomarkers. Pregnancy and neonatal outcomes will be collected and analysed. The first enrolment for the pilot study was in April 2014. As of April 2016, 26,670, women have been screened and 1760 recruited to the RCT.

Ethics & Dissemination This study will be conducted in accordance with the principles of Good Clinical Practice. This protocol was submitted to the National Research Ethics Committee and a favourable ethical opinion was granted. The reference number is 13/LO/1479 Subsequent approval by individual ethical committee and competent authority was granted. Results will be published in peer-reviewed journals and disseminated at international conferences.

The study is registered on the International Standard Randomised Controlled Trial Number (ISRCTN) registry.

Trial registration number: ISRCTN13633058
Date of primary registration: 28.10.2010
WHO UTN number: U1111-1140-4837

Background

Preeclampsia (PE) is an important cause of maternal and perinatal mortality and morbidity. There is extensive evidence that the risk of adverse outcome in relation to PE is much higher when the disease is severe and of early onset requiring delivery before 37 weeks' gestation (preterm-PE), than at term.[1-4] A major challenge in modern obstetrics is early identification of pregnancies at high-risk of preterm-PE and undertaking the necessary measures to improve placentation and reduce the prevalence of the disease.

Prediction of preterm preeclampsia

Extensive research in the last 20 years, mainly as a consequence of the shift in screening for aneuploidies from the second to the first-trimester of pregnancy, has identified a series of early biophysical and biochemical markers of impaired placentation.[5] A combination of maternal demographic characteristics, including medical and obstetric history, uterine artery pulsatility index (PI), mean arterial pressure (MAP) and maternal serum pregnancy associated plasma protein-A (PAPP-A) and placental growth factor (PlGF) at 11-13 weeks' gestation can identify a high proportion of pregnancies at high-risk for PE.[6] A recent study involving 58,800 singleton pregnancies examined at 11-13 weeks' gestation has further refined the prediction algorithm for PE. Using this algorithm the estimated detection rate of preterm-PE was 75%, at a false positive rate of 10%.[6]

Prevention of preterm preeclampsia

The prophylactic use of low-dose aspirin for prevention of PE has been an important research question in obstetrics for the last three decades. In 1979, Crandon and Isherwood observed that nulliparous women who had taken aspirin regularly during pregnancy were less likely to have PE than those who did not.[7] There have been two meta-analyses published reporting that the administration of low-dose aspirin in high-risk pregnancies is associated with a decrease in the rate of PE.[8,9] However, there are also other possible pathways which lead to the development of preeclampsia among different risk groups, and it is not known which risk factors or pathologic processes may be responsive to early initiation of low-dose aspirin.

Initiation of low-dose aspirin in early pregnancy

In most studies that evaluated aspirin for the prevention of PE, the initiation of treatment was at or after 16 weeks' gestation. Examination of a small number of randomised trials of low-dose aspirin in women at high-risk for PE suggests that the effectiveness of therapy is related to the gestational age at the initiation of treatment. A meta-analysis by Bujold *et al.* reported that low-dose aspirin started at 16 weeks' or earlier was associated with a significant reduction in the relative risk (RR) for PE (0.47, 95% confidence interval [CI] 0.34-0.65) and fetal growth restriction (FGR) (0.44, 95% CI 0.30-0.65).[9] In contrast, aspirin started after 16 weeks did not have a significant benefit (PE: RR 0.81, 95% CI 0.63-1.03; FGR: RR 0.98, 95% CI 0.87-1.10). More detailed analyses of these data on PE demonstrated that low-dose aspirin started at or before 16 weeks' gestation was particularly effective in preventing preterm-PE rather than term-PE (RR: 0.11, 95% CI 0.04-0.33 vs. RR: 0.98, 95% CI 0.42-2.33).[10]

The small number and small size of individual trials preclude definitive conclusions to be drawn regarding the effectiveness of aspirin starting before 16 weeks' and the results need to be examined in a prospective major randomised trial.

Aspirin resistance

There is evidence that approximately 30%, 10% and 5% of pregnant women are "aspirin resistant" with dosage of 81 mg, 121 mg, and 162 mg, respectively.[11] Furthermore, a retrospective cohort study reported that women who were identified by the PFA-100 test as being resistant to 81 mg of aspirin were less likely to develop severe PE when the dose of aspirin was increased from 81 to 162 mg, compared to those who continued with 81 mg.[12] Consequently, a trial investigating the effectiveness of low-dose aspirin in the prevention of preterm-PE should use a dose closer to 160 mg than 80 mg.

Safety of low-dose aspirin

The relative safety of first-trimester use of low-dose aspirin has been demonstrated in large cohort and case-control studies, which reported that the drug is not associated with increase in risk of congenital heart defects or other structural or developmental anomalies.[13-16]

Randomised studies reported that approximately 10% of women receiving low-dose aspirin complained of gastro-intestinal symptoms; however there was no evidence of increase in any type of maternal bleeding.[17-19] Similarly, the best evidence suggests that low-dose aspirin started before 16 weeks' gestation does not increase the risk of placental abruption (RR: 0.62, 95% CI 0.08–5.03).[9] No additional adverse effects related to epidural anaesthesia have been reported in women taking low-dose aspirin compared to those taking placebo.[20]

Prospective and case-control studies did not find an association between daily consumption of 60-150 mg of aspirin during the third-trimester and antenatal closure of the ductus arteriosus.[21-23] A meta-analysis including more than 26,000 women randomised to low-dose (80-150 mg) aspirin or placebo/no treatment during pregnancy demonstrated that the use of aspirin was not associated with an increase in intra-ventricular haemorrhage or other neonatal bleeding.[24] On the basis of currently available evidence it would be reasonable to continue with low-dose aspirin well into the third-trimester of pregnancy.

Hypothesis

We hypothesise that prophylactic low-dose aspirin administered from first-trimester of pregnancy in women at increased risk for preterm PE will reduce the incidence and severity of the disease.

Aim

To examine if the prophylactic use of low-dose aspirin administered from the first-trimester of pregnancy in women at increased risk for preterm PE can reduce the incidence and severity of the disease.

Objectives

Primary objective

To determine the efficacy of low-dose aspirin (150 mg daily), given to high-risk women from 11-14 weeks' gestation until 36 weeks', in reducing the incidence of preterm-PE, requiring delivery before 37 weeks.[25]

Secondary objectives

- To determine the effect of low-dose aspirin on adverse outcome of pregnancy at <37 weeks.
  - PE requiring delivery at <37 weeks'
  - SGA (<5th percentile) requiring delivery at <37 weeks'
  - Miscarriage or stillbirth at <37 weeks'
  - Placental abruption (clinically or on placental examination) at <37 weeks'
  - Composite of any of the above
- To determine the effect of low-dose aspirin on adverse outcome of pregnancy at <34 weeks.
  - PE requiring delivery at <34 weeks'
  - SGA (<5th percentile) requiring delivery at <34 weeks'
  - Miscarriage or stillbirth at <34 weeks'
  - Placental abruption (clinically or on placental examination) at <34 weeks'
  - Composite of any of the above
- To determine the effect of low-dose aspirin on adverse outcome of pregnancy at ≥37 weeks.
  - PE requiring delivery at ≥37 weeks'
  - SGA (<5th percentile) requiring delivery at ≥37 weeks'
  - Miscarriage or stillbirth at ≥37 weeks'
  - Placental abruption (clinically or on placental examination) at ≥37 weeks'
  - Composite of any of the above
- To determine the effect of low-dose aspirin on neonatal mortality and morbidity.
  - Neonatal intensive care unit admission
  - Intraventricular haemorrhage (IVH) grade II or above - Defined as bleeding into the ventricles
    - *Grade II (moderate)* – IVH occupies <50% of the lateral ventricle volume
    - *Grade III (severe)* – IVH occupies >50% of the lateral ventricle volume
    - *Grade IV (severe)* – Haemorrhagic infarction in periventricular white matter ipsilateral to a large IVH
  - Ventilation - Defined as need of positive pressure (continuous positive airway pressure (CPAP) or nasal continuous positive airway pressure (NCPAP)) or intubation
  - Neonatal sepsis - Confirmed bacteraemia in cultures
  - Anaemia – Defined as low haemoglobin and / or haematocrit requiring blood transfusion
  - Respiratory distress syndrome - Defined as need of surfactant and ventilation as a result of prematurity
  - Necrotising enterocolitis (NEC) requiring surgical intervention
    NEC is defined by a combination of clinical, radiological and laboratory features:
    - *Systemic signs* - apnoea, bradycardia, temperature instability, hypotension.
    - *Intestinal signs* - abdominal distension, gastric residuals, bloody stools, absent bowel sounds, abdominal tenderness, peritonitis.

        - *Radiological signs* - pneumatosis intestinalis or portal venous air, pneumoperitoneum.
        - *Laboratory changes* - metabolic and or respiratory acidosis, thrombocytopaenia, DIC.
    - Composite of any of the above
- To determine the effect of low-dose aspirin on the incidence of neonatal birthweight below the 3rd, 5th and 10th centile.
    - Birthweight will be recorded in the participants' medical notes and birthweight percentile for gestational age at delivery is calculated using a normal range derived from our population.[26]
- To determine the effect of low-dose aspirin on the incidence of stillbirth or neonatal death.
    - Due to any cause
    - Ascribed to PE or FGR
    - In association with maternal or neonatal bleeding
- To determine the effect of low-dose aspirin on the incidence of spontaneous preterm delivery at <34 weeks and <37 weeks.
    - Spontaneous delivery at <34 weeks (early preterm) and at <37 weeks (total preterm) includes those with spontaneous onset of labour and those with preterm pre-labour rupture of membranes.

Centres

There are thirteen academic hospitals participating in the trial. There are six centres in the United Kingdom, three in Spain, and one in each of Milan, Brussels, Greece and Israel.

Design

There are three components to the study: an internal pilot study, a screening quality study and a screening study followed by a double-blinded randomised placebo-controlled trial. Informed consent will be obtained by a trained healthcare professional who is a member of the study team at each particular centre.

Internal pilot study

The main study has been preceded by a two-month pilot study, undertaken at King's College Hospital. 1,106 participants have been consented into the screening study and 56 participants to the RCT. This pilot study has been used to assess the feasibility of recruitment to both the screening study and RCT and the ability of the centre to ensure successful compliance. A review by the ASPRE Independent Data Monitoring Committee (IDMC) and Trial Steering Committee (TSC) of the internal pilot study has demonstrated the study has been successful with respect to recruitment to both the screening study and RCT; however it has also highlighted the complexity of the main ASPRE trial and confirmed the need for enhanced quality systems to be in place in advance of starting the main ASPRE trial in order to ensure the quality of pivotal data. □

Screening Quality Study

A screening quality study, with a minimum recruitment period of one to three months (dependent on sites' performance) at each site, has been introduced to precede the main ASPRE trial. The aim of this study is to establish systems that will monitor quality of the measurement of uterine artery PI, MAP, PAPP-A and PlGF in a more detailed, formalised

manner at sites and use these systems to assess quality, identify areas for improvement and, where required, implement strategies to improve quality e.g. re-training. This is based on the DQASS system that has been successful for improving the quality of the ultrasound and biochemical measurements in the NHS fetal anomaly screening programme.

Recruitment rates have also been monitored. Furthermore, an assessment of data quality was made by the trial team at UCL CCTU and any site-specific operational issues, which could not have been foreseen by the site assessment process, were identified, and addressed in advance of starting the main ASPRE trial.

Screening Study and Randomised Control Trial

Following receipt of the result of the screening study, eligible high-risk women will be invited to take part in the RCT by designated the trial teams. It is anticipated that 10% of the population will screen positive for preterm-PE and be invited to participate in the trial (Figure 1).

Inclusion and exclusion criteria

Screening phase inclusion criteria

- Age ≥ 18 years;
- Singleton pregnancy;
- Live fetus at 11-13 weeks' of gestation;
- English, Italian, Spanish, French, Dutch or Greek speaking (otherwise interpreters will be used);
- Informed and written consent.

Screening phase exclusion criteria

- Multiple pregnancy;
- Pregnancies complicated by major fetal abnormality identified at the 11-13 weeks assessment;
- Women who are unconscious or severely ill, those with learning difficulties, or serious mental illness;
- Age < 18 years.

Randomisation inclusion criteria following screening

- Screening phase inclusion criteria fulfilment;
- High-risk for preterm-PE at 11-13 weeks' by the algorithm combining maternal history and characteristics, biophysical findings (MAP and uterine artery PI) and biochemical factors (PAPP-A and PlGF).

Randomisation exclusion criteria following screening

- Women taking low-dose aspirin regularly;
- Bleeding disorders such as Von Willebrand's disease;
- Peptic ulceration;
- Hypersensitivity to aspirin or already on long term non-steroidal anti-inflammatory medication;
- Concurrent participation in another drug trial or at any time within the previous 28 days;

- Any other reason the clinical investigators think will prevent the potential participant from complying with the trial protocol.

Methods

We will recruit women attending for their routine first scan in pregnancy at 11-13 weeks' gestation in the UK, Spain, Belgium, Italy, Greece and Israel. All eligible women attending for their routine first scan in pregnancy at 11-13 weeks' gestation are invited to take part. For the screening quality study and the screening study the PIS will be sent with the appointment letter to all potential participants.

In women who agree to participate in the screening quality study, after obtaining informed consent, we will date the pregnancy by the crown-rump length, [27] measure the maternal MAP an automated device,[28] use transabdominal colour Doppler ultrasound to visualise the left and right uterine artery and measure the PI in each vessel and calculate the mean PI.[29] Maternal serum PlGF is measured in the same blood sample taken for the measurement of PAPP-A, using automated machines that provide reproducible results (DELFIA Xpress system, PerkinElmer Life and Analytical Sciences, Waltham, USA) as part of the routine screening for Down's syndrome. Participants enrolled in the screening quality study will not be informed of their risk of developing PE and will be managed according to routine standard of care at the site they attend. The Principal Investigators at each site are doctors who received their training by Professor Nicolaides and follow the Fetal Medicine Foundation (FMF) guidelines on how to undertake the appropriate measurements.

In women who agree to participate in the screening study of the main trial, after obtaining informed consent, we measure maternal MAP, uterine artery PI, PAPP-A and PlGF as described above. Following screening for preterm-PE, high-risk women will be invited to take part in the RCT by designated members of the trial teams. Women eligible to participate in this trial will receive written information on the test drug and provide informed consent. When randomised, participants will be assigned a randomisation code. The randomisation codes will determine who receives placebo or aspirin 150 mg. The IMP supplier, Mawdsley Brooks and Co. will keep and store the randomisation code list. All participants, the PI and clinical trial pharmacy will remain blind to trial drug allocation.

Data Collection

Participant data for this study will be entered into an electronic case report form (CRF). For participants in the RCT that will be printed and signed by the enrolling researcher.

Randomisation

Randomisation will be performed using a web-based system Sealed Envelope. The website randomly assigns participants to a randomisation code which correspond to treatment packs with the same code at a given site. Each treatment pack will only be identified by a randomisation code. The treatment allocation will only be revealed to the researchers after completion of the study or where clinically essential.

Concealment of allocation

Mawdsley Brooks and Co. will provide labelling (for all cartons and blister sheets) ensuring complete blinding of the investigational medicinal product (IMP) to all investigators and participants in the study. That includes the PI, participating research doctors, pharmacists at the local clinical trial pharmacy, project managers and others involved in the trial. They are

all blinded to the IMP allocation. Matching placebo tablets will be identical to the intervention (aspirin) in such parameters as size, thickness, physical properties and appearance. A film coating will be applied to the placebo tablets for aesthetic and taste reasons.

Mawdsley Brooks and Co. will keep the randomisation code list confidential to maintain the blind, however the randomisation code list will be transferred to Sealed Envelope to enable to online randomisation and unblinding service to be established.

Intervention

Participants will take one tablet per night of either aspirin 150 mg or matched placebo. Participants will be asked to stop taking tablets at 36 weeks' gestation or, in the event of early delivery, at the onset of labour (maximum duration of 25 weeks). The aspirin tablets will be film-coated, to be taken orally once per night from enrolment until 36 weeks' gestation.

Study assessment

The study procedure by visit has been outlined in table 1.

Laboratory Tests

At the time of the 11-13 weeks scan, 20 mL of maternal blood will be taken for the measurement of PAPP-A and PlGF using automated machines that provide reproducible results (DELFIA Xpress system, PerkinElmer Life and Analytical Sciences, Waltham, USA). The remaining serum and plasma will be stored at -80^{o}C for future studies of potential biochemical markers for adverse pregnancy outcomes.

Participant compliance

Participants will be asked to bring their trial medication to each clinical visit; IMP compliance will be assessed by trial teams by counting remaining tablets at each follow up visit and asking about compliance at telephone follow up. Compliance with other aspects of the trial protocol will also be assessed. Participants will be encouraged to report any concerns or side effects in a diary for review at each trial visit.

Outcomes

Primary outcome

- Incidence of preterm-PE (delivery at <37 weeks)

PE will be defined as per the International Society for the Study of Hypertension in Pregnancy.[30] The systolic blood pressure should be 140 mm Hg or more and/or the diastolic blood pressure should be 90 mmHg or more on at least two occasions four hours apart developing after 20 weeks of gestation in previously normotensive women (blood pressure less than 140/90 mmHg) and there should be proteinuria of 300mg or more in 24 hours or

urinary protein creatinine ratio of 30 mg/mmol or more or two readings of at least ++ on dipstick analysis of midstream or catheter urine specimens if no 24-hour collection is available. The efficacy will be assessed by the development of PE at any gestation after 20 weeks of pregnancy as defined above. In preeclampsia superimposed on chronic hypertension, significant proteinuria (as defined above) should develop after 20 weeks' gestation in women with known chronic hypertension (history of hypertension before conception or the presence of hypertension at the booking visit at <20 weeks' gestation in the absence of trophoblastic disease).

Secondary outcomes

- As defined above in the secondary objectives section.

Collection of pregnancy and neonatal outcomes

Data on pregnancy and neonatal outcomes will be collected from the hospital maternity records or their general medical practitioners. The obstetric records of the randomised women with pre-existing or pregnancy-associated hypertension will be examined to determine if the condition was chronic hypertension, PE or gestational hypertension. Gestational hypertension will be classified as BP >140 (systolic) or 90 (diastolic) mmHg without proteinuria that occurs after 20 weeks gestation.

In the event neonates are admitted to Special Care Baby Unit (SCBU), additional neonatal outcomes will be collected from the discharge summary of SCBU.

Side effects and adverse events reporting

Adverse event (AE) and reaction (AR) data are not being collected for participants of the screening quality study, or the screen-negative participants in main ASPRE RCT, as they are non-CTIMPs which do not expose participants to any additional risk over and above that of routine clinical care.

Safety evaluations will be conducted at each of the RCT participants' follow-up visits. Adverse events include any unwanted side effects, sensitivity reactions, abnormal laboratory results, injury or inter-current illnesses, and may be expected or unexpected. The period for AE reporting will be from the time of first dose until 30 days post final IMP administration. The participants will be followed up by a telephone interview 30 days after the last dose of IMP. These AEs will be recorded on the electronic CRF and do not need to be reported to the Sponsor. The participants are instructed to contact a member of the trial team if there are any concerns regarding their medication.

Serious adverse events/reactions (SAE/SAR) occurring in the mother or baby from the time a participant is randomised until 30 days after stopping taking the IMP or until 30 days after delivery or until 30 days after the estimated due date, respectively, whichever is later, will be reported to the Sponsor using the trial documentation. The standard definition of a SAE will be used.[31]

For the purposes of this study the following events are included as protocol defined exceptions to SAE reporting should only be reported to the Sponsor as an SAE/SAR if the

investigator believes the event is a result of the ASPRE intervention: hospitalisation for maternal or fetal observation, including minor bleeding episodes; preterm delivery (spontaneous, for maternal or fetal indication); miscarriage; stillbirth or neonatal death; admission of baby to Neonatal Intensive Care Unit; termination for fetal or maternal indication. If the event is deemed to be part of the routine progress of the pregnancy concerned, these events should be reported to the Sponsor as a protocol defined exception to SAE reporting, within the respective reporting timelines.

Statistical analysis plan including sample size and power calculation

The sample size calculation is based on a 76% detection rate of the first-trimester combined screening for preterm-PE at a screen positive rate of 10%.[6] With the aim to achieve a significant 50% reduction in the prevalence of preterm-PE from 7.6% in the placebo group to 3.8% in the aspirin group, with a power of 90%, and 5% significance level, it is necessary to randomise 1,600 high-risk pregnancies. If we allow for 10% loss to follow up, it will be necessary to randomise a total of 1,760 high-risk pregnancies, 880 women in each of the aspirin and placebo arms.

Type of analysis and statistical tests

The primary analysis will comprise an intention-to-treat comparison of the two groups with respect to the proportion of high-risk pregnancies that develop preterm-PE at the two tailed 5% level. 95% confidence intervals will be produced for the proportions developing preterm PE in each of the two groups and for the difference (active – placebo).

Planned secondary analysis of the primary outcome will include a survival analysis of the time to delivery with PE treating births for other causes as censoring. Pre-specified baseline variables considered to be predictive will be included as appropriate. Their interactions with the treatment effect will be investigated. Gestational age at randomisation and its interaction with treatment will also be investigated. This analysis of treatment interactions will be considered as exploratory.

Descriptive statistics

A full set of descriptive statistics for all variables, overall and by treatment group, will be produced. Graphical displays will be produced as appropriate.

Secondary Analysis

Secondary outcomes will be compared across treatment groups using appropriate tests. P values and 99% confidence intervals will be produced for treatment effects. No corrections will be made for multiplicity.

Safety

The incidence rates of adverse events and serious adverse events and their relationship to trial drugs will be summarized by treatment group. The proportion of women discontinuing treatment will be summarized by reason and by treatment group.

Committee Oversights

The IDMC is independent from the trial and is responsible for monitoring the progress of the trial including: recruitment, protocol adherence, SAEs and side effects of treatment as well

as the difference between the trial treatments on the primary outcome measures. They are the only oversight body that has access to unblinded accumulating comparative data. The IDMC is responsible for safeguarding the interests of trial participants, monitoring the accumulating data and making recommendations to the TSC on whether the trial should continue as planned.

The TSC is the independent group responsible for oversight of the trial in order to safeguard the interests of trial participants. The TSC provides advice to the Chief Investigator, Co-Chief Investigator, UCL CCTU, the funder and sponsor on all aspects of the trial through its independent Chair.

Ethics and dissemination

The study will be conducted in accordance with the principles of Good Clinical Practice. A favourable ethical opinion was obtained from London-Fulham Research Ethics Committee, reference number 13/LO/1479. Subsequent approval by individual ethical committee and competent authority was granted. Results will be published in peer-reviewed journals and disseminated at international conferences.

Discussion

The traditional approach to screening for PE is to identify risk factors from maternal demographic characteristics and medical history, but such an approach can identify only 35% of total PE and about 40% of preterm-PE at false-positive rate of about 10%.[25,32]

In a proposed new approach to antenatal care, the potential value of an integrated clinic at 11-13 weeks' gestation in which maternal characteristics and history are combined with the results of a series of biophysical and biochemical markers to assess the risk for a wide range of pregnancy complications has been extensively documented.[33] Effective screening for preterm-PE can be achieved in this clinic with a detection rate of about 76% at a false-positive rate of 10%.[6] There is a suggestion that the prevalence of PE can be halved by prescribing pregnant women low-dose aspirin before 16 weeks' gestation[9] and by using an enhanced screening approach utilising maternal demographics and history with both biochemical and biophysical markers, these women can be identified effectively and entered into a double-blinded randomised placebo-controlled trial to assess whether low-dose aspirin can truly reduce the prevalence of preterm-PE when given in the first-trimester of pregnancy.

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Figure legend

Figure 1. Flow chart of participants in the Screening Study and the Randomised Control Trial. *Clinical visits at 19-24 weeks and 30-37 weeks will only be performed on screen-negative participants at sites where a scan is performed by the fetal medicine unit as part of the routine clinical care pathway at either of these times.

Table 1: Summary of the study visits

	Screening Visit	Randomisation Visit	First telephone interview	First follow up visit	Second telephone interview	Second follow up visit	Third follow up visit	Third telephone interview
Gestation (weeks)	11-13	11-14	16	19-24	28	32-34	36	30 days after the last dose of IMP
Patient information and characteristics	√							
Informed consent	√	√						
Measurement of weight and height	√			√		√	√	
Measurement of MAP	√			√		√	√	
Fetal ultrasound scan	√			√		√	√	
Measurement of uterine artery PI	√			√		√	√	
Measurement of PAPP-A and PlGF	√			√		√		
Check concomitant medications		√	√	√	√	√	√	
IMP dispensing		√		√				
Ensure compliance			√	√	√	√	√	
Check side effects/adverse events and review of diary card			√	√	√	√	√	√
Discontinue IMP							√	

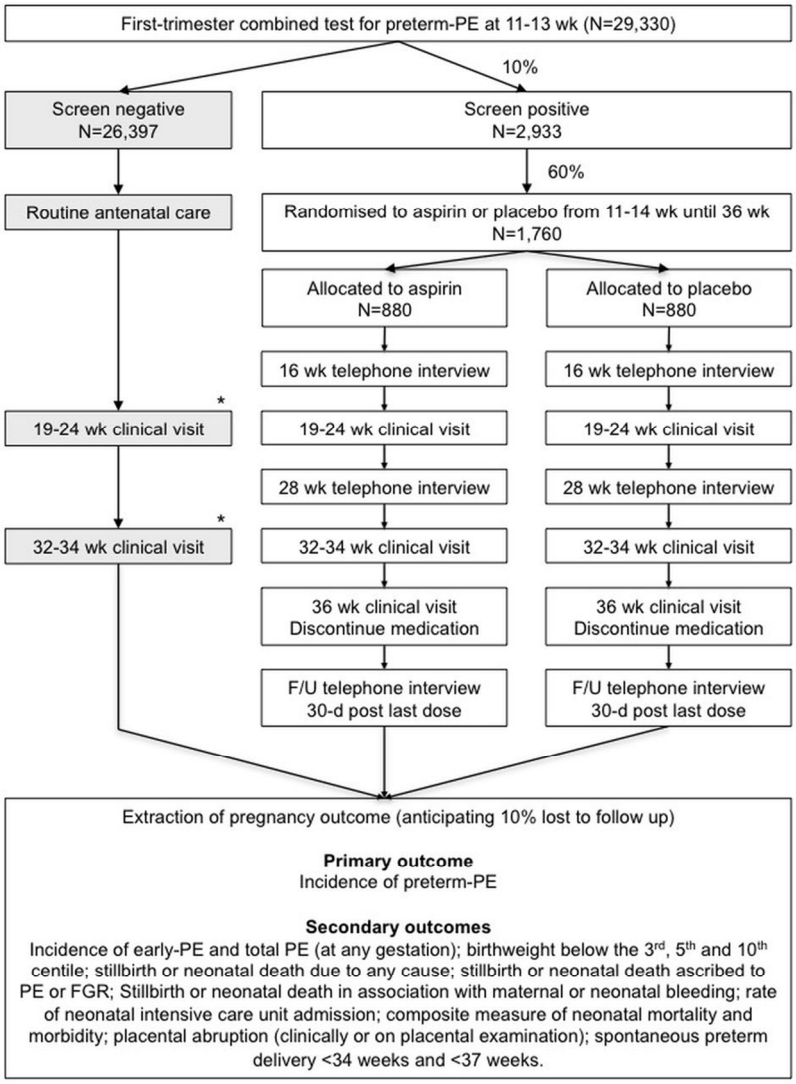

Figure 1. Flow chart of participants in the Screening Study and the Randomised Control Trial. *Clinical visits at 19-24 weeks and 30-37 weeks will only be performed on screen-negative participants at sites where a scan is performed by the fetal medicine unit as part of the routine clinical care pathway at either of these times.
90x119mm (300 x 300 DPI)

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
	2b	All items from the World Health Organization Trial Registration Data Set	1,2,3,4,7,8,9,10,11
Protocol version	3	Date and version identifier	3
Funding	4	Sources and types of financial, material, and other support	1
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1
	5b	Name and contact information for the trial sponsor	2
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	1
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	12

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4,5
	6b	Explanation for choice of comparators	9
Objectives	7	Specific objectives or hypotheses	5,6,7
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	7
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	7
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	8,9
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	10
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	11
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	7,8
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	8,10
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	10,11
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	10,18 & figure 1

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	11
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	8
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	9
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	9
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	9
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	9
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	9
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	10,11
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	10,11

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	9
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	11,12
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	12
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	Not applicable
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	12
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	12
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	11
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	6,14
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	3
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	Not applicable

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	8
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	Not applicable
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	9
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	1
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	2
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	Not applicable
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	3
	31b	Authorship eligibility guidelines and any intended use of professional writers	1
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	Not applicable
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	8
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	10

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.