SPREE
Screening programme for pre-eclampsia

Version	2.0
Date	31 May 2016
Sponsor	King's College London (KCL)
UCL Clinical Trials Unit	CTU/2014/102
Trial #	
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MREC #	15/LO/2161

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Date 31 May 2016

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Date 31 May 2016

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1 Administrative information

This document was constructed using the UCL Comprehensive Clinical Trials Unit (UCL CCTU) Protocol template Version 2.0. It describes the SPREE study, sponsored by KCL and co-ordinated by UCL CCTU.

It provides information about procedures for entering participants into the study, and provides sufficient detail to enable: an understanding of the background, rationale, objectives, study population, methods, statistical analyses, ethical considerations, dissemination plans and administration of the study; replication of key aspects of study methods and conduct; and appraisal of the study's scientific and ethical rigour from the time of ethics approval through to dissemination of the results. Every care has been taken in drafting this protocol, but corrections or amendments may be necessary. These will be circulated to registered investigators in the study. Sites entering participants for the first time should confirm they have the correct version through a member of the study team at UCL CCTU.

1.1 Compliance

The study will be conducted in compliance with the approved protocol, the Declaration of Helsinki (2008), the principles of Good Clinical Practice (GCP) as laid down by the Commission Directive 2005/28/EC with implementation in national legislation in the UK by Statutory Instrument 2004/1031 and subsequent amendments, the UK Data Protection Act, and the National Health Service (NHS) Research Governance Framework for Health and Social Care (RGF). International sites will comply with the principles of GCP as laid down by ICH topic E6 (Note for Guidance on GCP), Commission Directive 2005/28/EC, the European Directive 2001/20/EC (where applicable) and other national and local applicable regulations. Agreements that include detailed roles and responsibilities will be in place between participating sites and UCL CCTU.

1.2 Sponsor

KCL is the study sponsor and has delegated responsibility for the overall management of the SPREE study to UCL CCTU. Queries relating to KCL sponsorship of this study should be addressed to the Director of UCL CCTU, or via the study team.

1.3 Structured study summary

Source of Monetary or Material Support	National Institute for Health Research Efficacy and Mechanism Evaluation (EME) programme
Primary Sponsor	King's College London
Secondary Sponsor	Sponsor responsibilities for study management are delegated to UCL CCTU by the regulator, primary sponsor KCL.
Contact for Public Queries	ctu.spree@ucl.ac.uk
Contact for Scientific Queries	Dr Liona Poon Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, London, SE5 9RS, UK. Telephone: +44 7795312884 Fax: +44 20 3299 3898 Email: chiu_yee_liona.poon@kcl.ac.uk Prof Kypros Nicolaides Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, London, SE5 9RS, UK. Telephone: +44 2032998256 Fax: +44 20 3299 3898 Email: kypros@fetalmedicine.com
Public Title	Screening programme for pre-eclampsia (SPREE)
Scientific Title	Screening programme for pre-eclampsia
Countries of Recruitment	United Kingdom
Health Condition(s) or Problem(s) Studied	Pre-eclampsia (PE)
Screening	All women with singleton pregnancies undergoing routine 11-13 weeks scan will be invited to participate in the screening study for PE.
Key Inclusion and Exclusion Criteria	**Inclusion criteria** • Age $\geq$ 18 years, • Singleton pregnancies, • Live fetus at 11-13 weeks of gestation, • Informed and written consent. **Exclusion criteria** • Women who are unconscious or severely ill, those with learning difficulties or serious mental illness. • Pregnancies complicated by major fetal abnormality identified at 11-13 weeks of gestation
Study Type	Multicentre prospective cohort study
Date of First Enrolment	April 2016
Target Sample Size	16,850
Primary Outcome(s)	The primary outcome will be the false positive and true positive frequencies for screening for PE using the Bayes theorem based method and for screening according to the NICE guidelines. The screen positive threshold applied to the risks will be determined by the NICE guidelines. It is anticipated that there will be an increase in detection rate for all-PE from 35% (NICE method) to 50% (mini-combined test), at false positive rate of 10%.

Key Secondary Outcomes	Secondary outcome measures:
	
	• To demonstrate an increase in detection rate for all-PE:
	  ○ From 35% (NICE method) to 55% (combined test), at false positive rate of 10%;
	• To demonstrate an increase in detection rate for preterm-PE:
	  ○ From 40% (NICE method) to 60% (mini-combined test), at false positive rate of 10%.
	  ○ From 40% (NICE method) to 75% (combined test), at false positive rate of 10%.

1.4 Roles and responsibilities

1.4.1 Protocol contributors

Name	Affiliation	Role
Liona Poon	Harris Birthright Research Centre for Fetal Medicine, King's College Hospital.	Chief Investigator
Kypros Nicolaides	Harris Birthright Research Centre for Fetal Medicine, King's College Hospital.	Co-Chief Investigator
David Wright	University of Exeter Medical School, Royal Cornwall Hospital, Truro.	Study statistician

1.4.2 Role of study sponsor and funders

Name	Affiliation	Role
National Institute for Health Research Efficacy and Mechanism Evaluation (EME) programme	N/A	Scientific peer review of the study proposal Provision of funds Study monitoring
King's College London (KCL)	N/A	Sponsor
The Comprehensive Clinical Trials Unit at UCL (UCL CCTU)	UCL	All Sponsor responsibilities delegated to UCL CCTU by KCL.

1.4.3 Study Team

Name	Affiliation	Role and responsibilities
Liona Poon	Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, UK.	Chief Investigator Responsible for the concept and design of the study protocol, application for ethics and R&D approval, coordination and management of the study, statistical analysis of data, and writing up the scientific publications.
Kypros Nicolaides	Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, UK.	Co-Chief Investigator Responsible for the concept and design of the study protocol, supervision of the research team for the measurement of biomarkers, coordination and management of the study, monitoring and statistical analysis of data, and writing up the scientific publications.
Kate Maclagan	UCL CCTU	Clinical Project Manager Responsible for the project management of the study and oversight of the UCL CCTU team members
Harriet Quartly	UCL CCTU	UCL CCTU Study Manager Responsible for the day-to-day management of the study.
Details of site PIs are available in a separate document, outside of the study protocol.		

1.4.4 Study Management Group

Name	Affiliation	Role and responsibilities
Liona Poon	Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, UK.	Chief Investigator Responsible for overall coordination, day-to-day management of the study, monitoring of data.
Kypros Nicolaides	Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, UK.	Co-Chief Investigator Responsible for overall coordination and management of the study, monitoring of data.
Min Yi Tan	Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, UK.	Clinical Research Fellow Responsible for day-to-day study coordination at the study sites and data collection
David Wright	University of Exeter Medical School, Royal Cornwall Hospital, Truro.	Statistician Responsible for statistical analysis and monitoring of data.
Kate Maclagan	UCL CCTU	Clinical Project Manager Responsible for the project management of the study and oversight of the UCL CCTU team members
Harriet Quartly	UCL CCTU	UCL CCTU Study Manager Responsible for the day-to-day management of the study

1.4.5 Study Steering Committee

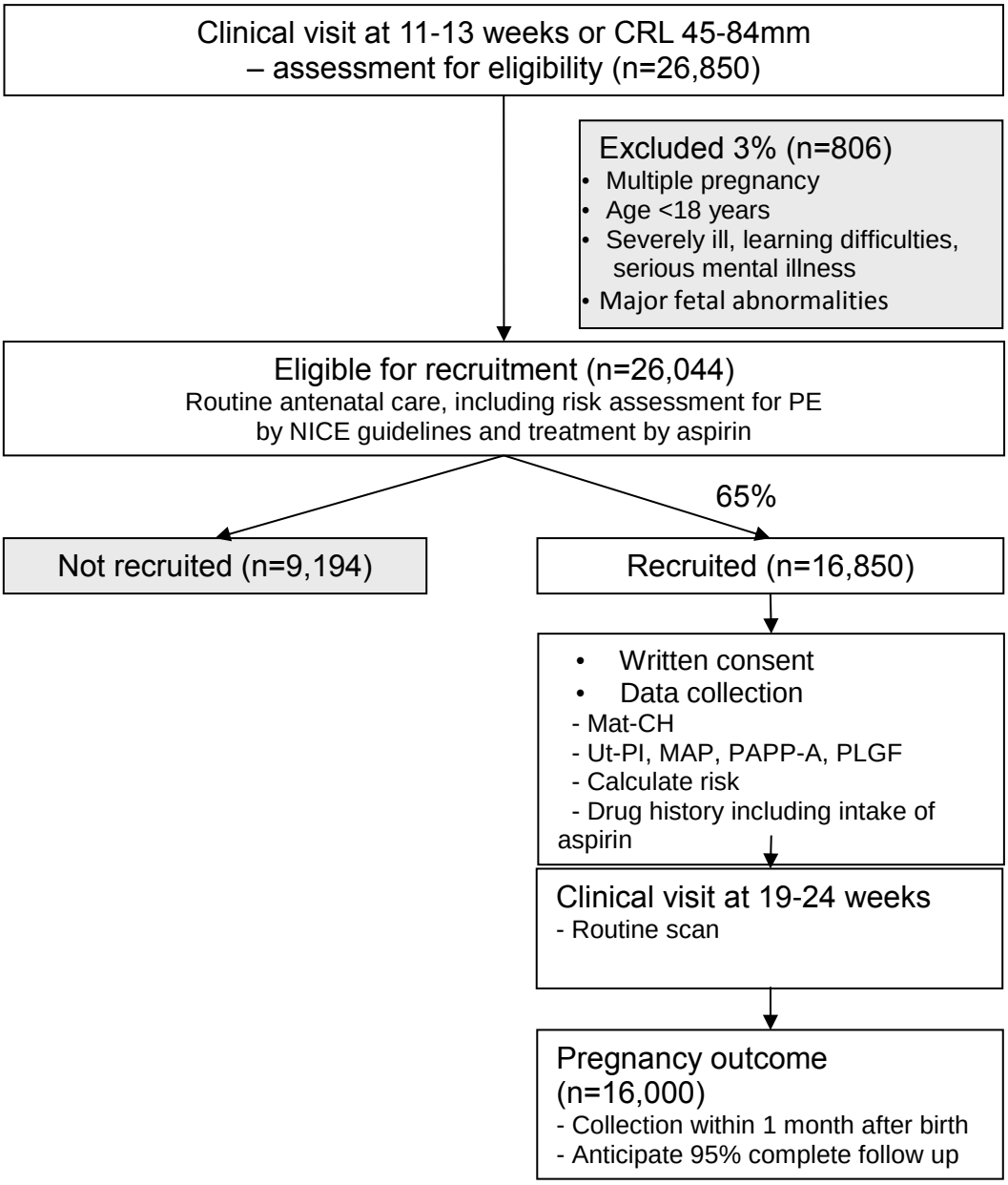
Name	Affiliation	Role and responsibilities
Zarko Alfirevic	Institute of Translational Medicine University of Liverpool, UK.	Chair Independent of the study team
Liona Poon	Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, UK.	Chief Investigator
Kypros Nicolaides	Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, UK.	Co-Chief Investigator
Jane Fisher	Antenatal Results Choices	PPI representative
Mehali Patel	BLISS	PPI representative
Ian Bradbury	Statistics at Frontier Science Scotland, UK.	Independent Statistician Responsible for monitoring and statistical analysis data.

1.4.7 SPREE Partners

Name	Role and responsibilities

Perkin Elmer (Wallac Oy), Finland	Perkin Elmer will be responsible for the production and supply of PlGF reagents.
Roche	Roche will be responsible for the production and supply of PlGF reagents.
Thermo Fisher Scientific	Thermo Fisher Scientific will be responsible for the production and supply of PlGF reagents.

2 Study Diagram



CRL = Crown-Rump Length; MAP = mean arterial pressure; PI = pulsatility index; PAPP-A = pregnancy associated plasma protein-A; PlGF = placental growth factor

3 Abbreviations

ß-hCG	ß-human chorionic gonadotropin
CI	Chief Investigator
CI	Confidence interval
CRF	Case Report Form
CRL	Crown-rump length
CTU	Clinical Trials Unit
FMF	Fetal Medicine Foundation
GCP	Good Clinical Practice
ICH	International Conference on Harmonisation
IDMC	Independent data monitoring committee
IRB	Institutional Review Board
KCL	King's College London
MAP	Mean arterial pressure
Mat-CH	Maternal characteristics and medical history
NREC	National Research Ethics Committee
NT	Nuchal translucency
PAPP-A	Pregnancy associated plasma protein-A
PI	Principal Investigator
PlGF	Placental growth factor
PIS	Participant Information Sheet
PET	Pre-eclampsia
QA	Quality Assurance
QC	Quality Control
QP	Qualified person
REC	Research Ethics Committee
RR	Relative risk
SAP	Statistical Analysis Plan
SSA	Site Specific Approval
SMF	Study Master File
SMG	Study Management Group
SMT	Study Management Team
ToR	Terms of Reference
SSC	Study Steering Committee
UCL	University College London
Uterine artery PI	Uterine artery pulsatility index

4 Glossary of Terms

Adverse Outcome - a harmful outcome that is usually indicated by some result such as morbidity, mortality.

Algorithm - a formula or set of steps for solving a particular problem.

Aneuploidy - an abnormal number of chromosomes.

Cohort study - a study with two or more groups of people - cohorts - with similar characteristics. One group receives a treatment, is exposed to a risk factor or has a particular symptom and the other group does not. The study follows their progress over time and records what happens.

Crown-Rump length - is the measurement of the length of human embryos and fetuses from the top of the head (crown) to the bottom of the buttocks (rump).

Detection rate – the frequency of discovering a certain outcome from an analysis.

False positive rate - the frequency of a result that indicates that a given condition is present when it is not.

Fetus - the name for an embryo after 8 weeks of development

Hypertension (high blood pressure) - is a chronic medical condition in which the blood pressure in the arteries is elevated.

Impaired placentation – occurs when the placenta does not establish an adequate blood supply from the uterus.

Meta-analysis - a statistical method which can be used to combine the results of two or more studies.

Nuchal translucency - the thickness at the back of an unborn baby's neck. Measuring this thickness helps assess the risk of Down's syndrome and other abnormalities.

Patient demographics – they are objective characteristics of a population; e.g. age, marital status, family size, racial origin, present or prior disease, religion, income, and education.

Perinatal – refers to the period immediately before and after birth.

Placentation – the formation, type and structure or arrangement of placentas

Pre-eclampsia – a multisystem disorder of pregnancy associated with high blood pressure and proteinuria.

Prophylactic use – the use of a medication or a treatment designed to prevent a disease from occurring.

Prospective study - watches for outcomes, such as the development of a disease, during the study period and relates this to other factors such as suspected risk or protection factor(s).

Pulsatility index - a measure of the variability of blood velocity in a vessel.

Randomised control trial - a study in which a number of similar people are randomly assigned to two (or more) groups to test a specific drug or treatment. One group (the experimental group) receives the treatment being tested, the other (the comparison or control group) receives an alternative treatment, a dummy treatment (placebo) or no treatment at all. The groups are followed up to see how effective the experimental treatment was.

Relative risk - the ratio of the risk of disease or death among those exposed to certain conditions compared with the risk for those who are not exposed to the same conditions. If both groups face the same level of risk, the

relative risk is 1. If the first group had a relative risk of 2, participants in that group would be twice as likely to have the event happen. A relative risk of less than one means the outcome is less likely in the first group.

Screening – investigations that aim to find serious health conditions early, before any symptoms develop.

Uterine artery Doppler scan – ultrasound assessment of the blood flow in the vessels that supply the uterus.

5 Introduction

5.1 Background and Rationale

Background:

Existing research:

Current recommendation of screening for PE

PE, which affects about 2% of pregnancies, is a major cause of maternal and perinatal morbidity and mortality. PE can be subdivided into preterm-PE, requiring delivery <37 weeks' gestation and term-PE with delivery ≥37 weeks. Preterm-PE is associated with a higher incidence of adverse outcome. The current guideline from the National Institute for Health and Clinical Excellence (NICE) recommends that at the booking visit, women at high-risk or with more than one moderate risk factor for PE should be identified and they should be advised to take low-dose aspirin daily from 12 weeks until the birth of the baby [1]. The performance of the current method of screening is poor and identifies only about 40% of the women that develop preterm-PE and 35% of all-PE.

Prevention of PE by low-dose aspirin

The evidence of benefit from prophylactic use of low-dose aspirin is derived from meta-analyses of randomised studies, which reported a reduction in the prevalence by about 10% [2]. There is also some evidence from some studies on small number of cases suggesting that the beneficial effect of aspirin in women at increased risk of PE may be substantially increased if treatment is initiated before 16 weeks' gestation [3-5]. The extent to which this is true is currently being investigated by our multicentre study (CI Nicolaides; ASPRE study funded by the FP7 of the European Commission), which is anticipated to report results by early 2017.

Prediction of PE

Extensive studies in the last decade have established that the best performance for early prediction of PE can be achieved by a combination of maternal characteristics and medical history (Mat-CH) together with the measurements of the mean arterial pressure (MAP), uterine artery pulsatility index (PI), serum placental growth factor (PlGF) and serum pregnancy associated plasma protein-A (PAPP-A) at 11-13 weeks' gestation (combined test) [6]. A combination of maternal factors, MAP and PAPP-A forms the mini-combined test.

We developed a new method of first-trimester screening for PE based on prospectively derived data from 60,000 pregnancies. The method uses a novel survival-time model based on Bayes' theorem to combine the prior information from Mat-CH with the biomarker multiple of the median (MoM) to estimate patient-specific risk for development of PE [7,8]. This model has now been finalised based on data from 120,000 pregnancies. In the new model, the gestational age at the time of delivery for PE is treated as a continuous rather than categorical variable, offering the option to clinicians and researchers to select their own gestational age cut-off to define the high-risk group that could potentially benefit from therapeutic interventions starting from the first trimester.

The four biomarkers used in the model have been extensively investigated and are readily available for clinical use. Research by our group has led to the development of protocols for standardised and auditable measurements of MAP, uterine artery PI, PAPP-A and PlGF [9-12]. Reproducible measurements of PAPP-A and PlGF can be undertaken using automated platforms that are currently used for provision of screening for Down's syndrome in all maternity hospitals in the England.

Our research has also established that the performance of screening depends on firstly, characteristics of the study population (including racial group and prior history of PE), and secondly, adjustment of measurements of biophysical and biochemical tests for maternal and pregnancy characteristics (expressed as multiple of median [MoM). Extensive experience of Professor David Wright, who is responsible for Quality Assurance for the UK Down's syndrome screening programme of the National Screening Committee, has highlighted firstly, the

importance of training and audit in the use of biophysical tests and secondly, need for monitoring median levels and adjustment of values of biochemical tests according to reagents provided by different manufacturers.

The first trimester combined test for PE screening, using a novel Bayes-based method, is likely to be superior to the current method recommended by NICE based on Mat-CH. The estimated detection rates, for false positive rate of 10%, of the NICE method are about 40% for preterm-PE and 35% for all-PE and the respective values for our combined test and mini-combined test are 75% and 55%, and 60% and 50%. Though the performance of the mini-combined test is lower, it is cheaper with no additional biochemical markers above standard of care.

This study is necessary to prospectively validate the proposed new tests for the screening of PE.

5.2 Objectives

The primary aim of the study is to compare screening for PE using a Bayes theorem based method with screening using current NICE guidelines. The reference standards will be PE defined according to the International Society for the Study of Hypertension in Pregnancy [13] and the American College of Obstetricians and Gynecologists [14]. If a participant fulfils the criteria for PE by one definition and not the other they will be considered to have developed PE.

The objectives of the study are:

- To evaluate the performance of the new method of screening for PE, which uses Bayes theorem to combine the prior information from Mat-CH with biomarker levels to estimate the patient-specific risk for PE leading to delivery before any pre-specified gestational age, compared to that of the current method recommended by the NICE. We anticipate that the new method of screening will substantially improve the early detection of PE in the first trimester of pregnancy and that this method will be such so that any potentially useful new biomarkers identified in the future would be easily incorporated into the algorithm. Quality assurance of biophysical and biochemical markers used in screening for PE will be continuously monitored. The primary outcome of this study is to demonstrate a significant improvement in the detection rate for PE with the use of the proposed screening method, as compared to that of the existing guidelines.
- To improve our understanding of the pathophysiology of PE, according to the severity of the condition. PE is thought to be the consequence of impaired placentation leading to placental hypoxia and release of inflammatory factors. These cause platelet and endothelial cell activation leading to the development of the clinical signs of the disease. We will investigate biomarker profiles in pregnancies complicated by PE of different severity, as reflected in the gestational age at delivery and neonatal birth weight, from data we have already collected longitudinally at 11-13 weeks' gestation (n=120,000), 20-24 weeks (n=65,000) and 30-34 weeks (n=30,000).

5.3 Study Design

This is a prospective cohort study in at least six NHS hospitals.

<u>Multicentre study comparing performance of an alternative method for screening for pre-eclampsia with that used currently (NICE)</u>

To achieve results representative of what would happen in practice, a single gate multicentre prospective, cohort study of 16,850 pregnancies in at least six centres will be undertaken.

Training of healthcare professionals in participating centres:
We will undertake training for recording of Mat-CH, performing measurement of MAP [9] and uterine artery PI [10], collecting and analysing blood for PAPP-A and PlGF, calculating patient-specific risk for PE and recording pregnancy outcome to ensure consistency across sites.

Maternal serum concentrations of PAPP-A and PlGF are measured using either the DELFIA XPRESS analyser (PerkinElmer Life and Analytical Sciences, Waltham, USA), BRAHMS KRYPTOR analyser (Thermo Fisher Scientific, Hennigsdorf, Germany) or Cobas analyser (Roche Diagnostics, Penzberg, Germany).

Clinical evaluation of new screening method:
The study will be coordinated by the Comprehensive Clinical Trials Unit at UCL (UCL CCTU). Our method will be examined in at least six NHS maternity hospitals. In these hospitals, all eligible women attending for their routine 11-13 weeks scan will be invited to participate. Where possible, the patient information sheet will be sent with the appointment letter to all potential participants. On arrival for the 11-13 weeks scan, eligible women will be counselled by a dedicated researcher or midwife and those who agree will provide written informed consent. The Mat-CH will be recorded, the MAP and uterine artery PI measured according to standardised protocols. Maternal blood will be collected for measurements of serum PAPP-A and PlGF according to standardised protocols. Risk for PE will be calculated.

In participating hospitals, the basis of recommending the use of aspirin is the NICE guidelines but the degree of adherence to these guidelines is expected to be variable. The results of the combined test for PE will not be provided to the patients or their obstetrician and midwives and these will not be used to influence management. We will record whether the participants are taking aspirin or not at the 11-13 week scan. The participants will make a hospital visit at 19-24 weeks for a second ultrasound examination as part of their routine care. Quality assurance of biomarkers will be undertaken on a monthly basis and each site will be given feedback according to the results generated by the quality assurance algorithm.

Mechanistic components of the study:
PE is thought to be the consequence of impaired placentation leading to placental hypoxia and release of inflammatory factors. These cause platelet and endothelial cell activation leading to the development of the clinical signs of the disease.

We will evaluate the longitudinal biomarker profiles (MAP, uterine artery PI, PlGF) in pregnancies complicated by PE, according to the severity of the condition as reflected in the gestational age at delivery and neonatal birth weight; from data we have already collected longitudinally at 11-13 (n=120,000), 20-24 (n=65,000) and 30-34 (n=30,000) weeks' gestation.

We will use samples from our existing bio-bank to continue our research into the pathophysiology of PE and discovery of new biomarkers. We have carried out metabolomics studies in maternal serum at 11-13 weeks' gestation and found significant changes from normal in a number of metabolites in pregnancies with PE, fetal trisomies and congenital cardiac defects [15,16]. We are validating these metabolomics markers to ascertain the impact on diagnostic accuracy when they are combined with our established biomarkers.

The study will be conducted in compliance with the protocol, the Declaration of Helsinki (1996), the principles of Good Clinical Practice (GCP). The study will be reviewed and approved by the National Research Ethics Committee (NREC) as well as applicable Hospital Trusts. The University College London Comprehensive Clinical Trials Unit (UCL CCTU) will manage the sponsors' responsibilities and Quality Assurance to ensure compliance with GCP.

6 Methods

6.1 Site Selection

The study sponsor has overall responsibility for site and investigator selection.

6.1.1 Study Setting
This is a multicentre study that will be carried out in the Fetal Medicine Units in the UK (that are within the FMF Research Network).

6.1.2 Site/Investigator Eligibility Criteria

Once a site has been assessed as being suitable to participate in the study, the study team will provide them with a copy of this protocol and relevant study documents.

To participate in the SPREE study, investigators and study sites must fulfil a set of criteria that have been agreed by the SPREE Study Management Group (SMG) and that are defined below.

Study sites meeting eligibility criteria and that are accepted by the SMG as being suitable to recruit to the study, will be issued with the SPREE Study Master File (SMF) documentation to use when applying for Site-Specific Approval (SSA).

6.1.2.1 Principal Investigator's (PI) Qualifications and Agreements

The investigator(s) must be willing to sign a UCL CCTU Clinical Study Site Agreement or an Investigator Agreement to comply with the study protocol (confirming their specific roles and responsibilities relating to the study, and that their site is willing and able to comply with the requirements of the study). This includes confirmation of appropriate qualifications, agreement to comply with the principles of GCP, to permit monitoring and audit as necessary at the site, and to maintain documented evidence of all staff at the site who have been delegated significant study related duties.

6.1.2.2 Resourcing at site

The investigator(s) should be able to demonstrate a potential for recruiting the required number of suitable participants within the agreed recruitment period (i.e. the investigator(s) regularly treat(s) the target population). They should also have an adequate number of qualified staff and facilities available for the foreseen duration of the study to enable them to conduct the study properly and safely.

Sites will be expected to complete a delegation of responsibilities log and provide staff contact details.

The site should have sufficient data management resources to allow prompt data return to UCL CCTU.

6.2 Site approval and activation

On receipt of the signed Clinical Study Site Agreement, Investigator Agreement, approved delegation of responsibilities log and staff contact details, written confirmation will be sent to the site PI. The study manager or delegate will notify the PI in writing of the plans for site initiation.

The site must conduct the study in compliance with the protocol as agreed by the Sponsor and, which was given favourable opinion by the Research Ethics Committee (REC) and/or Institutional Review Board (IRB). The PI or delegate must document and explain any deviation from the approved protocol, and communicate this to the study team at UCL CCTU.

A list of activated sites may be obtained from the Study Manager.

6.3 Participants

6.3.1 Eligibility Criteria

6.3.1.1 Participant selection

There will be **NO EXCEPTIONS** (waivers) to eligibility requirements at the time of recruitment. Questions about eligibility criteria should be addressed PRIOR to attempting to recruit the participant.

The eligibility criteria for this study have been carefully considered and are the standards used to ensure that only medically appropriate participants are entered. Participants not meeting the criteria should not be entered into the study. It is therefore vital that exceptions are not made to these eligibility criteria.

Participants will be considered eligible for enrolment in this study if they fulfil all the inclusion criteria and none of the exclusion criteria as defined below.

6.3.1.2 Participant Inclusion Criteria

- Age $\geq$ 18 years;
- Singleton pregnancies;
- Live fetus at 11-13 weeks of gestation;
- Informed and written consent.

6.3.1.3 Participant Exclusion Criteria

- Women who are unconscious or severely ill, those with learning difficulties or serious mental illness.
- Pregnancies complicated by major fetal abnormality identified at 11-13 weeks of gestation

6.3.1.4 Eligibility Criteria for Individuals Performing the Interventions

All centres involved in the data collection will have staff who are appropriately trained in obstetric ultrasound and possess certificates of competence from the FMF for non-routine measurements.

6.3.1.5 Co-enrolment Guidance

For parous women, participation in the study in a previous pregnancy will be checked (as there will be a record in the electronic Source Data) in order to prevent participants from being enrolled more than once in this study. Data of each participant should only be recorded as one entry in the database.

6.3.1.6 Recruitment Procedures

Written informed consent will be obtained from all women agreeing to participate in the screening study for PE and **BEFORE** any study-specific procedures are performed for the study. The only procedures that may be performed in advance of written informed consent being obtained are those that would be performed on all participants in the same situation as part of usual standard of care, such as the routine first-trimester combined screening for aneuploidies, which includes an ultrasound scan to measure fetal CRL, NT and assessment of fetal anatomy and blood draw for measurement of biochemical markers including PAPP-A (DELFIA XPRESS analyser [PerkinElmer Life and Analytical Sciences, Waltham, USA]; BRAHMS KRYPTOR analyser [Thermo Fisher Scientific, Hennigsdorf, Germany]; Cobas analyser [Roche Diagnostics, Penzberg, Germany]).

6.4 Outcomes

6.4.1 Primary Outcomes

The primary outcome will be the false positive and true positive frequencies for screening using the Bayes theorem based method and for screening according to the NICE guidelines. The screen positive threshold applied to the risks will be determined by the NICE guidelines. It is anticipated that there will be an increase in detection rate for all-PE from 35% (NICE method) to 50% (mini-combined test), at false positive rate of 10%.

The definitions of PE were that of the International Society for the Study of Hypertension in Pregnancy [13] and the American College of Obstetricians and Gynecologists [14]. The systolic blood pressure should be $\geq$140 mm Hg and/or the diastolic blood pressure should be $\geq$90 mmHg on at least two occasions four hours apart developing

after 20 weeks' gestation in previously normotensive women and there should be proteinuria (≥300 mg in 24 hours or two readings of at ≥2+ on dipstick analysis of midstream or catheter urine specimens if no 24-hour collection is available). In the absence of proteinuria, new onset of any of the following systemic findings: a) thrombocytopaenia (platelet counts <100,000 µL); b) renal insufficiency (creatinine >1.1 mg/dL or 2-fold increase in creatinine in the absence of underlying renal disease); c) abnormal liver function (ie, hepatic transaminase levels twice normal); d) pulmonary oedema; or e) cerebral or visual symptoms. Preterm-PE is PE that requires delivery before 37 weeks' gestation.

6.4.2 Secondary Outcomes

Secondary outcome measures:

- To demonstrate an increase in detection rate for all-PE:
  - From 35% (NICE method) to 55% (combined test), at false positive rate of 10%;
- To demonstrate an increase in detection rate for preterm-PE
  - From 40% (NICE method) to 60% (mini-combined test), at false positive rate of 10%.
  - From 40% (NICE method) to 75% (combined test), at false positive rate of 10%.

Collection of pregnancy and neonatal outcomes

Data on pregnancy outcomes will be collected from the hospital maternity records or their general medical practitioners.

The obstetric records of the participating women with pre-existing or pregnancy associated hypertension will be examined to determine if the condition was chronic hypertension, PE or gestational hypertension.

6.5 Participant Timeline

Study procedure by visit:

Screening Visit (11-13 week of pregnancy or CRL 45-84mm)

- Informed Consent
- Patient demographics
- Height and Weight
- Maternal medical and obstetric history
- Family history
- Drug history including aspirin intake
- Routine first-trimester scan
- MAP
- Uterine artery blood flow (transabdominal colour Doppler ultrasound)
- PAPP-A and PlGF measurements
- Risk calculation

Follow up visit: 19-24 week of pregnancy

- Routine anomaly scan

	11-13 weeks scan	**19-24 weeks scan**
Gestation (weeks)	11-13 or CRL 45-84mm	19-24
Patient information and characteristics	√	
Informed consent	√	
Measurement of weight and height	√	
Measurement of MAP	√	
Fetal ultrasound scan	√	√
Measurement of uterine artery PI	√	
Measurement of biochemical markers (PAPP-A and PlGF)	√	
Risk calculation	√	
Drug history including aspirin intake	√	

Laboratory Tests

At the time of the 11-13 weeks scan, 20mL of maternal blood will be taken for the measurement of biochemical markers using either the DELFIA XPRESS analyser (PerkinElmer Life and Analytical Sciences, Waltham, USA), BRAHMS KRYPTOR analyser (Thermo Fisher Scientific, Hennigsdorf, Germany) or Cobas analyser (Roche Diagnostics, Penzberg, Germany). At sites with available storage facilities, the remaining serum and plasma will be stored at -80^{o}C for future studies on potential biochemical markers of pregnancy complications.

6.5.1 Early Stopping of Follow-up

If a participant chooses to withdraw from the study, they should continue to be followed up according to the follow-up schedule defined in the protocol, providing they are willing. They should be encouraged not to leave the study. If, however, the participant exercises the view that they no longer wish to be followed up either, this view must be respected and the participant withdrawn entirely from the study. UCL CCTU should be informed of the withdrawal in writing using the appropriate SPREE study documentation. Unless consent for all data already collected is withdrawn it will be kept and included in analyses for all participants who stop follow up early.

Participants who stop study follow-up early will not be replaced.

6.5.2 Participant Transfers

If a participant moves from the area which is served by the hospital where they were recruited to the study, making continued follow up at their consenting centre inappropriate, every effort should be made for them to be followed at another participating study centre. Written consent should be taken at the new centre and then a copy of the participant's CRFs should be provided to the new centre. Responsibility for the participant remains with the original consenting centre until the new consent process is complete.

6.5.3 Loss to Follow-up

For participants that are lost to follow up, every effort should be made to contact their general practitioners in order to acquire the pregnancy outcomes. We will also use NHS spine to trace them by NHS number in case they change their GP.

6.5.4 Study Closure

The end of the study for individual participants will be defined as delivery of the baby/end of the pregnancy. The end of the study as a whole will be defined as the point at which the last participant (n=16,850) has fulfilled the above definition and details of their complete pregnancy outcome have been collected. This will take approximately 15 months to complete.

6.6 Sample Size

We propose to recruit 16,850 patients from at least six UK centres (Table 1). On the assumption of a 5% no follow up rate there will be 16,000 evaluable patients.

Table 1. Recruitment centres with average number of deliveries per year and anticipated number of patients that would be recruited to the study.

Recruitment centre	Average deliveries/year	Anticipated recruitment
King's College Hospital	11,000	5,100
Medway Maritime Hospital	5,500	2,600
North Middlesex Hospital	5,500	2,600
Homerton University Hospital	6,000	2,800
Southend University Hospital	3,900	1,875
University Hospital Lewisham	3,900	1,875
Total	**35,800**	**16,850**

The power properties of the primary analysis across a range of assumptions regarding the effect of aspirin were examined using computer simulations each of 100,000 trials. The power properties of the test depend on the effectiveness of aspirin and the proportions of patients treated with aspirin. The most pessimistic situation, in terms of power to detect a difference in sensitivity is where a large proportion of patients in the NICE screened positive group are treated and a small proportion in the NICE screened negative group are treated and aspirin is most effective. The power properties of the study under the most extreme case are illustrated in Table 2. The test has power in excess of 80% to detect differences of 10 percentage points.

Table 2. Power for the detection of difference in sensitivity between the index text and the comparator at the one sided 2.5% level with a sample of 16,000 evaluable patients. The figures in the body of the table are the power of the test. The margins give the sensitivity of the comparator test and the index text. It is assumed that 90% of NICE screened positive patients and 10% of NICE screened negative patients are treated with aspirin and that aspirin reduces the incidence of PE by 50%.

		Comparator								
		35%	**40%**	**45%**	**50%**	**55%**	**60%**	**65%**	**70%**	**75%**
	35%		33.4%	85.0%	99.3%	100.0%	100.0%	100.0%	100.0%	100.0%
	40%			32.0%	84.1%	99.2%	100.0%	100.0%	100.0%	100.0%
	45%				31.7%	83.7%	99.1%	100.0%	100.0%	100.0%
Index test	**50%**					31.8%	83.9%	99.2%	100.0%	100.0%
	55%						31.9%	84.8%	99.4%	100.0%
	60%							33.2%	86.5%	99.6%
	65%								34.8%	88.7%

6.7 Recruitment and Retention

6.7.1 Recruitment

In all the participating centres, all women attending for the routine 11-13 weeks scan will be invited to take part in the screening study for PE. Where possible, the PIS will be sent with the appointment letter to all potential participants.

Recruitment rates will be actively monitored by the SMG. This will include analyses by centre of the number of women recruited. Appropriate strategies will be implemented if recruitment falls below an acceptable level.

6.7.2 Retention

If women fail to attend their follow-up visits, the study coordinators will contact them to arrange for another visit within 7 days of the study visit window.

6.8 Data Collection, Management and Analysis

6.8.1 Data Collection Methods

Patient information for this study will be entered into an electronic CRF. The main CRF will be held electronically only and will not be printed. The CRF will be composed of 3 parts:

CRF part 1 This part will include patient demographics, medical, obstetric and drug history, family history, measurement of MAP, collection of blood samples for biochemical testing, performance of an ultrasound scan to confirm the GA by the measurement of fetal CRL and measurement of the uterine artery PI; and risk for PE. Participants will be identified on CRFs by initials, patient code and site name, date of enrolment and the enrolling site-PI or fellow.

CRF part 2 will record details of the routine clinical visits and medical, obstetric and drug history at 19-24 weeks.

CRF part 3 will record details of pregnancy outcomes, details of labour, development of PE, neonatal birth weight and outcomes.

If a participant withdraws from follow-up a paper CRF will be completed. CRFs must be kept current to reflect the participant's status at each phase during the course of the study.

The CRFs will be the source documents of the study that must be available at all times for audit.

A participant identification record will be kept by each site-PI that would allow linking of the participant study number, participant name and date of birth for those included in the study along with participant contact information. This file will be kept electronically at sites.

6.8.2 Non-Adherence and Non-Retention

For non-adherers (as defined by poor attendance to the clinical visit) we will record details of pregnancy outcomes, details of labour, development of PE, neonatal birth weight and outcomes as for adherers. Reasons for non-adherence and non-retention and those lost to follow up will be recorded in the CRF.

6.8.3 Data Management

The UCL CCTU will act as custodian for the study data. The following guidelines will be strictly adhered to:

- Patient data will be pseudo-anonymised.
- All anonymised data will be stored on a password protected UCL CCTU computer.

6.8.4 Statistical Methods

6.8.4.1 Statistical Analysis Plan

To enable the analyses to be reproduced and to produce the report in a timely way, the analysis will be programmed in R [17] in the period prior to the completion of follow up. It will be documented in a stand-alone statistical analysis plan (SAP). This will include all programmes, dummy tables and figures. The SAP will be finalised blinded to outcome data. Results will be presented according to the STARD guidelines. All data and programs will be provided to an independent statistician for evaluation.

6.8.4.2 Statistical Methods – Outcomes

The purpose of the analysis is to compare performance of screening using the Bayes theorem based method with that using the NICE criteria. The difference will be tested at the one-sided 2.5% level.

The primary analysis will be of the prospective cohort of 16,000 patients. All patients with data on maternal characteristics, medical history and outcome will be included in the analysis. Risks will be calculated using the algorithm developed. These calculations will be fully pre-specified so that the prospective cohort can be used as an independent test data set.

The essential features of our primary analysis are as follows. The NICE criteria define a high-risk group by applying a set of rules to information on Mat-CH resulting in a binary (positive/negative) outcome for each pregnancy. These results will provide an overall screen positive rate of around 10%. We will firstly determine the threshold for the risks that gives the same number of screen positives as NICE. Secondly, we shall construct the 2 x 2 contingency table of counts by classifying each patient with PE according to the result from NICE and the risk assessment (Table 3). We shall then apply an extension of McNemars test that uses the difference between the counts of discordant pairs b and c. Under the null hypothesis of equality, these counts have the same expected value. Under the alternative hypothesis of superiority of the Bayes theorem based method to NICE, the expected value of c exceeds that of b.

Table 3: Classification of cases PE according to screening results.

		Bayes method	
		+	**-**
NICE	**+**	a	b
	-	c	d

A complication for any comparative study of PE is that, by converting true positives to false positives, treatment with aspirin reduces the incidence of PE in the treated group. If treatment is dependent on screening using a particular method, then the bias will reduce the apparent DR for that method. We have therefore developed a modification to McNemars test that incorporates an assumed relative risk for aspirin and individual level data on aspirin treatment. We will be classifying individuals according to treatment with aspirin as summarised in Table 4.

Table 4: Classification of cases PE according to screening results.

Untreated

		Bayes method	
		+	-
NICE	+	a_0	b_0
	-	c_0	d_0

Treated

		Bayes method	
		+	-
NICE	+	a_1	b_1
	-	c_1	d_1

If aspirin reduces the incidence of PE by θ and the proportions of women in the population receiving aspirin are denoted by p_a, p_b, p_c and p_d in the cells corresponding to a, b, c and d then under the null hypothesis of no difference in DR the cell counts in Table 4 are to have expected values proportional to the probabilities in Table 5.

Table 5: Expected cell proportions according to treatment and test result.

Untreated

		Bayes method	
		+	-
NICE	+	$1-p_a$	$1-p_b$
	-	$1-p_c$	$1-p_d$

Treated

		Bayes method	
		+	-
NICE	+	$p_a(1-\theta)$	$p_b(1-\theta)$
	-	$p_c(1-\theta)$	$p_d(1-\theta)$

The modified test compares counts c_0+c_1 with b_0+b_1. Under the null hypothesis of no difference in sensitivity, the expected counts is in the ratio $1-p_c\theta : 1-p_b\theta$ respectively. Note that if $p_c = p_b$ or $\theta = 1$ (aspirin is ineffective) this ratio is 1:1 and the test reduces to McNemars test. If $\theta < 1$ (aspirin reduces risk) and $p_b > p_c$ then the expected value of the counts is larger for c_0+c_1 than for b_0+b_1 and McNemars test is biased against NICE. Our approach to analysis is to take an external estimate of θ based on the best available evidence at the time to adjust the analysis for the effect of aspirin. A sensitivity analysis with respect to variations across a plausible range of θ (e.g. a 95% confidence interval) based on the evidence at the time will be presented. Point and estimates and 95% confidence estimates of DR and differences between DR adjusted for potential treatment effects will also be produced using the model described above. The primary comparison, will be a comparison between NICE and the mini combined test.

Primary comparisons are as follows:

- NICE vs. the mini-combined test for all-PE.
- NICE vs. the combined test for all-PE;
- NICE vs. the mini-combined test for preterm-PE;
- NICE vs. the combined test for preterm-PE.

Secondary comparisons are as follows:

A. NICE vs. the Bayes risk assessment based on Mat-CH;
B. Bayes risk assessment using Mat-CH vs. mini-combined test;
C. Bayes risk assessment using Mat-CH vs. combined test;
D. Mini combined test vs combined test.

For comparisons (B) – (C) a receiver operating characteristic curve (ROC) analysis, including comparison of areas under the ROC curve will be reported. All comparisons will be made with respect to all-PE and preterm-PE.

6.8.4.2.1 Economic evaluations

Not applicable

6.8.4.3 Additional Analyses – Subgroup

There is evidence that a high detection rate of preterm-PE can be achieved by two-stage screening in the first-trimester with maternal factors and MAP in the whole population and measurements of uterine artery PI and PlGF in only some of the pregnancies (screened positive women identified through first-line screening). We also aim to prospectively evaluate contingent screening with maternal factors and MAP as the first-stage screening test in all pregnancies and reserving measurements of uterine artery PI and PlGF for the second-stage to only some of the population only for a subgroup of the population selected on the basis of the risk derived from screening by maternal factors and MAP alone.

6.8.4.4 Additional Analyses – Adjusted

The main study analyses is being adjusted for aspirin use, as described in section 6.8.4.2.

6.8.4.5 Analysis Population and Missing Data

Not applicable

6.9 Data Monitoring

6.9.1 Data Monitoring Committee

An Independent Data Monitoring Committee (IDMC) is not being convened for SPREE given the low-risk nature of the study. An Independent Statistician, who will review the statistical analysis plan is present on the SSC

6.9.2 Interim Analyses

N/A

6.9.3 Data Monitoring for Harm

This is a non-CTIMP and participants are not exposed to any additional risk over and above that of standard of care, therefore no safety reporting will be conducted for this study.

6.9.4 Quality Assurance and Control

6.11.4.1 Risk Assessment

The Quality Assurance (QA) and Quality Control (QC) considerations for the SPREE study are based on the standard UCL CCTU Quality Management Policy that includes a formal Risk Assessment, and acknowledges the risks associated with the conduct of the study and proposals of how to mitigate them through appropriate QA and QC processes. Risks are defined in terms of their impact on: the rights and safety of participants; project concept including study design, reliability of results and institutional risk; project management; and other considerations.

QA is defined as all the planned and systematic actions established to ensure the study is performed and data generated, documented and/or recorded and reported in compliance with the principles of GCP. QC is defined as the operational techniques and activities performed within the QA system to verify that the requirements for quality of the study related activities are fulfilled.

6.9.4.2 Central Monitoring at UCL CCTU

UCL CCTU staff will review Case Report Form (CRF) data for errors and missing key data points. The study database will also be programmed to generate reports on errors and error rates. Essential study issues, events and outputs, including defined key data points, will be detailed in the SPREE study Data Management Plan.

6.9.4.3 On-site Monitoring

The frequency, type and intensity of routine and triggered on-site monitoring will be detailed in the SPREE Quality Management and Monitoring Plan (QMMP). The QMMP will also detail the procedures for review and sign-off of monitoring reports.

6.9.4.3.1 Direct access to participant records

Participating investigators must agree to allow study-related monitoring, including audits and REC review, by providing access to source data and other study related documentation as required. Participant consent for this must be obtained as part of the informed consent process for the study.

6.9.4.4 Study Oversight

Study oversight is intended to preserve the integrity of the study by independently verifying a variety of processes and prompting corrective action where necessary. The processes reviewed relate to participant enrolment, consent, eligibility, and policies to protect participants, including reporting of harms; completeness, accuracy and timeliness of data collection; and will verify adherence to applicable policies detailed in the Compliance section of the protocol. Independent study oversight complies with the UCL CCTU study oversight policy.

In multi-centre studies this oversight is considered and described both overall and for each recruiting centre by exploring the study dataset or performing site visits as described in the SPREE Quality Management and Monitoring Plan.

6.9.4.4.1 Study Management Team

The Study Management Team (SMT) will be set up to assist with developing the design, co-ordination and day to day operational issues in the management of the study, including budget management. The membership, frequency of meetings, activity (including study conduct and data review) and authority will be covered in the SMT terms of reference.

6.9.4.4.2 Study Management Group

A Study Management Group (SMG) will be set up to assist with developing the design, co-ordination and strategic management of the study. The membership, frequency of meetings, activity (including study conduct and data review) and authority will be covered in the SMG terms of reference.

6.9.4.4.3 Study Steering Committee

The Study Steering Committee (SSC) is the independent group responsible for oversight of the study in order to safeguard the interests of study participants. The SSC provides advice to the CI, UCL CCTU, the funder and sponsor on all aspects of the study through its independent Chair. The membership, frequency of meetings, activity (including study conduct and data review) and authority will be covered in the SSC terms of reference.

6.9.4.4.4 Independent Data Monitoring Committee

An Independent Data Monitoring Committee (IDMC) is not being convened for SPREE given the low-risk nature of the study.

6.9.4.4.5 Study Sponsor

The role of the sponsor is to take on responsibility for securing the arrangements to initiate, manage and finance the study. KCL is the study sponsor and has delegated all Sponsor responsibilities to UCL CCTU.

7 Ethics and Dissemination

7.1 Research Ethics Approval

The study will be conducted in compliance with the principles of the Declaration of Helsinki (1996), the principles of GCP and in accordance with all applicable regulatory requirements including but not limited to the Research Governance Framework.

This protocol and related documents will be submitted for review to NREC.

Annual progress reports and a final report at conclusion of the study will be submitted to the UCL CCTU (on behalf of the Sponsor) and the NREC within the timelines defined in the Regulations.

Before initiation of the study at any clinical site, the protocol, all informed consent forms and any material to be given to the prospective participant will be submitted to the relevant REC for approval. Any subsequent amendments to these documents will be submitted for further approval. Before initiation of the study at each additional clinical site, the same/amended documents will be submitted for local Research and Development (R&D) approval.

The rights of the participant to refuse to participate in the study without giving a reason must be respected. After the participant has entered the study, the clinician remains free to give alternative treatment to that specified in the protocol, at any stage, if s/he feels it to be in the best interest of the participant. The reasons for doing so must be recorded. After recruitment the participant must remain within the study for the purpose of follow up and data analysis. However, the participant remains free to change their mind at any time about the protocol treatment and follow-up without giving a reason and without prejudicing their further treatment.

7.2 Other Approvals

The protocol will be submitted by those delegated to do so to the relevant R&D department of each participating site. A copy of the local R&D approval and of the Participant Information Sheet (PIS) and consent form on local headed paper must be forwarded to the co-ordinating centre before participants are recruited in the study.

The protocol has received formal approval and methodological, statistical, clinical and operational input from the UCL CCTU Protocol Review Committee.

7.3 Protocol Amendments

Substantial protocol amendments (e.g. changes to eligibility criteria, outcomes, sample size calculations, analyses) will be decided by the SMG. Each site-PI will be informed of the potential changes. Such amendments will be submitted to NREC for approval. Once approved, each site PI will be notified via email.

7.4 Consent or Assent

During the consent process it will be made completely and unambiguously clear that the participant is free to refuse to participate in all or any aspect of the study, at any time and for any reason, without incurring any penalty or affecting their treatment.

Consent will be re-sought if new information becomes available that affects the participant's consent in any way. This will be documented in a revision to the patient information sheet and the participant will be asked to sign an updated consent form. These will be approved by the ethics committee prior to their use.

A copy of the approved consent form is available from the UCL CCTU study team.

7.4.1 Consent or Assent in Ancillary Studies

Informed consent will be sought from participants for their remaining serum and plasma (following analysis of PAPP-A, free β-hCG and PlGF for risk assessments of aneuploidies and PE) to be stored at -80^{o}C for future studies of potential biochemical markers for pregnancy complications.

7.5 Confidentiality

Record of participants' demographic data, ultrasound scan and clinical findings and observations and biochemical data are routinely stored in one of the two commonly used password secured data management programmes in Obstetrics (astraia Obstetrics [astraia software gmbh, Munich, Germany] or ViewPoint [GE Healthcare gmbh, Solingen, Germany]). Participants will then be only identified by their patient codes. Access is limited to authorised study personnel (CI, Co-CI, site-PIs, database managers and statistician). They can only access the data with a password. This approach of data collection will enable collection of the complete patient records while maintaining confidentiality, so as to comply fully with the blanket requirement for anonymity of data.

7.6 Declaration of Interests

The investigators named on the protocol have no financial or other competing interests that impact on their responsibilities towards the scientific value or potential publishing activities associated with the study.

7.7 Archiving

The investigators agree to archive and/or arrange for secure storage of local SPREE study materials and records for indefinitely after the close of the study unless otherwise advised by the UCL CCTU.

7.8 Access to Data

Requests for access to study data will be considered, and approved in writing where appropriate, after formal application to the SMG/SSC. Considerations for approving access are documented in the SMG/SSC Terms of Reference.

7.9 Ancillary and Post-study Care

N/A

7.10 Publication Policy

7.10.1 Study Results

It is intended that the results of the study will be reported and disseminated at international conferences and in peer-reviewed scientific journals. The results of the study will be disseminated regardless of the direction of effect.

7.10.2 Reproducible Research

The study protocol will be published on SPREE study website (www.fetalmedicine.org).

8 Ancillary Studies

9 Protocol Amendments

Substantial Amendment 1	
1.	Pregnancies complicated by major fetal abnormality identified at 11-13 weeks of gestation have been added to the exclusion criteria
2.	The date of first enrolment has been changed to the actual month.
3.	The name of the Study Manager and an additional member of the SMG have been added.
4.	Clarification that drug history including aspirin intake is required at the 11-13 week visit but not at the 19-24 weeks visit
5.	No tablet count of aspirin is required.
6.	Clarification that where possible, patient information sheets will be sent with the appointment letter to potential participants
7.	Clarification that all study site staff involved in data collection will possess certificates of competence from the FMF for non-routine measurements.
8.	The number of deliveries expected at the study sites / year has been clarified to an average number/site
9.	Analysis of blood samples for β-hCG is not included in study specific procedures

10.	Maternal medical and obstetric history is not required at 19-24 weeks. A routine anomaly scan is carried out only at this time point.
11.	Additional study site(s) to the six listed in Table 1 may be added.

10 References

1. National Collaborating Centre for Women's and Children's Health (UK). Hypertension in Pregnancy: The Management of Hypertensive Disorders During Pregnancy. London: RCOG Press, 2010.
2. Askie LM, Duley L, Henderson-Smart DJ, Stewart LA; PARIS Collaborative Group. Antiplatelet agents for prevention of pre-eclampsia: a meta-analysis of individual patient data. Lancet 2007; 369: 1791-1798.
3. Bujold E, Roberge S, Lacasse Y, Bureau M, Audibert F, Marcoux S, Forest JC, Giguere Y. Prevention of preeclampsia and intrauterine growth restriction with aspirin started in early pregnancy: a meta-analysis. Obstet Gynecol 2010; 116: 402-414.
4. Roberge S, Villa P, Nicolaides K, Giguère Y, Vainio M, Bakthi A, Ebrashy A,Bujold E. Early administration of low-dose aspirin for the prevention of preterm and term preeclampsia: a systematic review and meta-analysis. Fetal Diagn Ther 2012; 31: 141-146.
5. Roberge S, Giguère Y, Villa P, Nicolaides K, Vainio M, Forest JC, von Dadelszen P, Vaiman D, Tapp S, Bujold E. Early administration of low-dose aspirin for the prevention of severe and mild preeclampsia: a systematic review and meta-analysis. Am J Perinatol 2012; 29: 551-556.
6. Poon LC, Kametas NA, Maiz N, Akolekar R, Nicolaides KH. First-trimester prediction of hypertensive disorders in pregnancy. Hypertension 2009; 53: 812-818.
7. Wright D, Akolekar R, Syngelaki A, Poon LC, Nicolaides KH. A competing risks model in early screening for preeclampsia. Fetal Diagn Ther 2012; 32: 171-178.
8. Akolekar R, Syngelaki A, Poon L, Wright D, Nicolaides KH. Competing risks model in early screening for preeclampsia by biophysical and biochemical markers. Fetal Diagn Ther 2013; 33: 8-15.
9. Poon LC, Zymeri NA, Zamprakou A, Syngelaki A, Nicolaides KH. Protocol for measurement of mean arterial pressure at 11-13 weeks' gestation. Fetal Diagn Ther 2012; 31: 42-48.
10. Plasencia W, Maiz N, Bonino S, Kaihura C, Nicolaides KH. Uterine artery Doppler at 11+0 to 13+6 weeks in the prediction of pre-eclampsia. Ultrasound Obstet Gynecol 2007; 30: 742-9.
11. Kagan KO, Wright D, Spencer K, Molina FS, Nicolaides KH. First trimester screening for trisomy 21 by free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A: impact of maternal and pregnancy characteristics. Ultrasound Obstet Gynecol 2008; 31: 493–502.
12. Pandya P, Wright D, Syngelaki A, Akolekar R, Nicolaides KH. Maternal serum placental growth factor in prospective screening for aneuploidies at 8-13 weeks' gestation. Fetal Diagn Ther 2012; 31: 87-93.
13. Brown MA, Lindheimer MD, de Swiet M, Van Assche A, Moutquin JM: The classification and diagnosis of the hypertensive disorders of pregnancy: Statement from the international society for the study of hypertension in pregnancy (ISSHP). Hypertens Pregnancy 2001; 20: IX-XIV.
14. American College of Obstetricians and Gynecologists; Task Force on Hypertension in Pregnancy. Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy. Obstet Gynecol 2013: 122: 1122-31.
15. Bahado-Singh RO, Akolekar R, Mandal R, Dong E, Xia J, Kruger M, Wishart DS, Nicolaides K. First-trimester metabolomic detection of late-onset preeclampsia. Am J Obstet Gynecol 2013; 208: 58.e1-7.
16. Bahado-Singh RO, Akolekar R, Mandal R, Dong E, Xia J, Kruger M, Wishart DS, Nicolaides K. Metabolomics and first-trimester prediction of early-onset preeclampsia. J Matern Fetal Neonatal Med 2012; 25: 1840-7.
17. R Development Core Team (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.