A study protocol for the prospective validation study:

Screening programme for pre-eclampsia (SPREE)

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Disclaimer

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

Provenance and peer review

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

Study oversight

The study is being coordinated by Harriet Quartly, the Study Manager, and Kate Maclagan, the Clinical Project Manager, the Comprehensive Clinical Trials Unit at University College London (UCL CCTU). King's College London is the study

sponsor. The UCL CCTU manages the sponsors' responsibilities and quality assurance to ensure compliance with GCP. They will act as custodian of the data and will have disclosure of contractual agreements.

Contributors

LCP, KHN and DW conceived and designed the study. LCP, KHN and DW 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, MYT, DW and KM drafted the original protocol. LCP and MYT drafted the manuscript. LCP, MYT with the support of HQ, the Study Manager and KM, the Clinical Project Manager, have responsibilities for day-to-day running of the study including participant recruitment, data collection and liaising with other sites. All authors critically reviewed and approved the final version of the manuscript.

Ethics approval

The study is conducted in compliance with the principles of the Declaration of Helsinki (1996), the principles of Good Clinical Practice (GCP) and in accordance with all applicable regulatory requirements including but not limited to the Research Governance Framework. A favorable ethical opinion was obtained from London-Surrey Borders Research Ethics Committee, reference number 15/LO/2161. Subsequent approval by individual ethical committee and competent authority was granted.

Dissemination

Results will be published in peer-reviewed journals and disseminated at international conferences.

Study Registration Number: ISRCTN83611527.

ABSTRACT

Introduction: Pre-eclampsia (PE), which affects about 2% of pregnancies, is a major cause of maternal and perinatal morbidity and mortality. Early detection of PE can improve pregnancy outcome by providing timely intervention and closer monitoring. The current guideline from the National Institute for Health and Care Excellence (NICE) recommends that at the booking visit, women identified with one major risk factor or more than one moderate risk factor for PE should be advised to take low-dose aspirin daily from 12 weeks until the birth of the baby. However, the performance of the current method of screening is poor and identifies only about 35% of PE. Extensive studies in the last decade have established that the best performance for early prediction of PE can be achieved by using a novel Bayes-based method that combines maternal characteristics and medical history together with the measurements of mean arterial pressure (MAP), uterine artery pulsatility index (PI), serum placental growth factor (PlGF) and pregnancy associated plasma protein-A (PAPP-A) at 11-13 weeks' gestation. This forms the "combined test", which could be simplified to the "mini-combined test" when only maternal factors, MAP and PAPP-A are taken into consideration

We present a protocol (Version 3.1, 14 November 2016) on the "Screening programme for pre-eclampsia" (SPREE) study.

Methods and analysis: This is a prospective multicenter cohort study carried out in seven NHS maternity hospitals in England. Eligible pregnant women attending for their routine scan at 11-13 weeks' gestation are invited to participate in this study. Maternal characteristics and history, and the measurements of MAP, uterine artery PI, serum PAPP-A and PlGF are recorded according to standardized protocols. The patient-specific risk for PE is calculated. Pregnancy outcomes will be collected. We hypothesize that the first-trimester mini-combined test and combined test for PE screening, using the Bayes-based method, are likely to be superior to the current method recommended by NICE that is based on maternal demographics and history alone.

The first enrolment for study commenced in April 2016. As of November, 13,000 women have been recruited. The study is registered on the International Standard Randomised Controlled Trial Number (ISRCTN) registry.

Background

Pre-eclampsia (PE) is a multi-system disorder characterized by hypertension and proteinuria in pregnancy. This condition affects about 2% of pregnancies and is a significant contributor to iatrogenic preterm birth. It remains a major cause of maternal and perinatal morbidity and mortality [1,2]. PE can be subdivided into preterm-PE, with delivery <37 weeks' gestation and term-PE with delivery $\geq$37 weeks. Preterm-PE is associated with a higher incidence of adverse short and long-

term outcomes [3-5].

<u>Purpose of screening</u>

An effective screening tool is essential to guide clinicians to correctly identify women at high-risk of developing PE. This subsequently allows early introduction of prophylactic treatment and therapeutic intervention and increased surveillance of such pregnancies.

The use of low dose aspirin in high-risk pregnant women has been shown in meta-analyses to confer a 10-17% risk reduction for PE [6,7]. Low-dose aspirin when commenced prior to 16 weeks' gestation has been demonstrated to potentially halve the prevalence of PE and fetal growth restriction, with a noted dose-response effect [8]. The extent to which this is true is currently being investigated by our multicenter randomized control trial of low-dose aspirin (150 mg daily) versus placebo, given to high-risk women from 11–14 week's gestation, in the reduction of incidence of preterm-PE (ASPRE study funded by the FP7 of the European Commission). The results are anticipated to be reported in 2017 [9].

<u>Current recommendation of screening for pre-eclampsia</u>

In the United Kingdom, the National Institute for Health and Care Excellence (NICE) recommends that at the antenatal booking visit, women should be risk stratified for developing PE based on their demographic characteristics and medical history [10]. Women with any single major risk factor or with more than one moderate risk factor should be advised to take low-dose aspirin daily from 12 weeks until the birth of the baby. The performance of the current method of screening at best identifies only

about 40% of the women who subsequently develop preterm-PE and 35% of all-PE, at a false-positive rate of 10% [11].

Improving pre-eclampsia prediction

Extensive studies in the last decade have established that the performance for early prediction of PE can be increased by the use of a Bayes-based method that combines maternal characteristics and medical history, with multiples of the median (MoM) of mean arterial pressure (MAP), uterine artery pulsatility index (PI), serum placental growth factor (PlGF) and pregnancy associated plasma protein-A (PAPP-A) at 11-13 weeks' gestation to estimate patient-specific risk for development of PE [12]. This forms the "combined test", which could be simplified to the "mini-combined test" when only maternal factors, MAP and PAPP-A are taken into consideration (Table 1). In the Bayes-based model, the gestational age at the time of delivery for PE is treated as a continuous rather than a 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 estimated detection rates for preterm-PE and all-PE, at a false-positive rate of 10%, of the combined test and mini-combined test are 75% and 55%, and 60% and 50%, respectively. Though the performance of the mini-combined test is lower, it is cheaper with no additional biochemical markers above standard care. The four biomarkers used in the model have been extensively investigated and are readily available for clinical use. Protocols have been developed for standardized and auditable measurements of MAP, uterine artery PI, PAPP-A and PlGF [13-16].

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 England.

Hypothesis

We hypothesize that the first-trimester mini-combined test and combined test for PE screening, using the Bayes-based method, are likely to be superior to the current method recommended by NICE that is based on maternal demographics and history alone.

Aim

The aim of this study is to prospectively validate the performance of the proposed new method of screening for PE and compare this to screening according to the NICE guidelines.

Objective

To evaluate the performance of the new method of screening for PE, which uses Bayes theorem to combine the prior information from maternal characteristics and medical history with biomarker levels to estimate the patient-specific risk for PE with delivery before any pre-specified gestational age, compared to that of the current method recommended by the NICE guidelines. 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.

Participating centers

The study is being carried out in seven NHS maternity hospitals in England that are within the Fetal Medicine Foundation Research Network:

- King's College Hospital

- Medway Maritime Hospital

- Southend University Hospital

- Homerton University Hospital

- North Middlesex University Hospital

- Universiy Hospital Lewisham

- Royal London Hospital

Study method

This is a prospective multicenter cohort study. The flow chart of the study method is illustrated in Figure 1. In participating hospitals, all eligible women attending for their routine 11-13 weeks' scan are invited to join the study. Where possible, the patient information sheet is sent with the appointment letter to all potential participants. On arrival for the 11-13 weeks' scan, eligible women are counseled and those who agree are requested for written informed consent. We collect data on maternal characteristics and medical history. The MAP and uterine artery PI are measured according to standardized protocols [13-14]. Maternal serum concentrations of PAPP-A and PlGF are measured using either the DELFIA XPRESS analyzer (PerkinElmer Life and Analytical Sciences, Waltham, USA), BRAHMS KRYPTOR analyzer (Thermo Fisher Scientific, Hennigsdorf, Germany) or Cobas analyser (Roche

Diagnostics, Penzberg, Germany) [15-16]. At sites with available storage facilities, the remaining serum and plasma will be stored at -80°C for future studies on potential biochemical markers of pregnancy complications.

The performance of the Bayes-theorem method of screening for PE [12] will be prospectively validated and compared to screening according to the NICE guidelines[10].

The basis of recommending the use of low-dose aspirin is as per the NICE guidelines but the degree of adherence to these guidelines is expected to be variable. The results of the mini-combined test and the combined test for PE are not provided to the participants or their clinicians to avoid any influence on routine management. Aspirin intake is recorded at the screening visit. Quality assurance of biomarkers is undertaken on a monthly basis and each site is given feedback according to the results generated by the quality assurance algorithm.

Participant eligibility

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

Inclusion criteria

- Age $\geq$ 18 years;
- Singleton pregnancies;
- Live fetus at 11-13 weeks' 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' gestation

Sample size

We propose to recruit 16,850 participants. On the assumption of a 5% no follow up rate, there will be 16,000 evaluable participants. 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 sTable 1. The test has power in excess of 80% to detect differences of 10 percentage points.

Outcomes

<u>Primary Outcomes</u>

- To calculate the false positive and true positive frequencies for screening using the Bayes' theorem based method and for screening according to the NICE guidelines.

- For all-PE, from 35% (NICE method) to 50% (mini-combined test), at false positive rate of 10%.

Secondary Outcomes

- 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%.

Pre-eclampsia definition

The definitions of PE were that of the International Society for the Study of Hypertension in Pregnancy [17] and the American College of Obstetricians and Gynecologists [18].

The systolic blood pressure should be >140 mm Hg and/or the diastolic blood pressure should be >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 defined as PE with delivery before 37 weeks' gestation.

Data collection

Patient information for this study will be entered into electronic Case Report Forms (CRFs).

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.

Statistical methods

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 [19] 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 programs, dummy tables and figures. The SAP will be finalized 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.

Statistical methods – outcomes

The purpose of the analysis is to compare performance of screening using the Bayes-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 participants. All participants 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 provided as supplementary materials.

Strengths and limitations

The strengths of this study include:

- The large sample size and multicenter approach, covering a wide selection of participants from various demographic backgrounds;
- Screening time period coincides with the gestational age for screening of chromosomal aneuploidies which would appeal to patients and care providers;
- Consistency in data collection by the provision of training for all investigators based on the Fetal Medicine Foundation protocols;
- Expression of the values of the biomarkers as MoMs after adjustment for factors that affect the measurements;

- The use of Bayes theorem to combine the prior risk from maternal factors with biomarkers to estimate patient-specific risks and the performance of screening for PE delivery at different stages of pregnancy.

The limitations of the study include:

- Not all obstetric units have access to sonographers trained in uterine artery Doppler studies or laboratories with placental biomarker assays, therefore the results of the study may not be widely implemented. Consequently, the mini-combined test is created as an alternative screening tool allowing the use of existing facilities with minimal requirements for further training;
- Follow-up of the neonates is limited to the early postnatal phase. However, this is adequate for the purpose of this study.

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Figure legends

Figure 1. Flow chart of study method. Key: crown rump length (CRL), mean arterial pressure (MAP), Uterine artery pulsatility index (Ut-PI), serum placental growth factor (PIGF) and serum pregnancy associated plasma protein-A (PAPP-A).
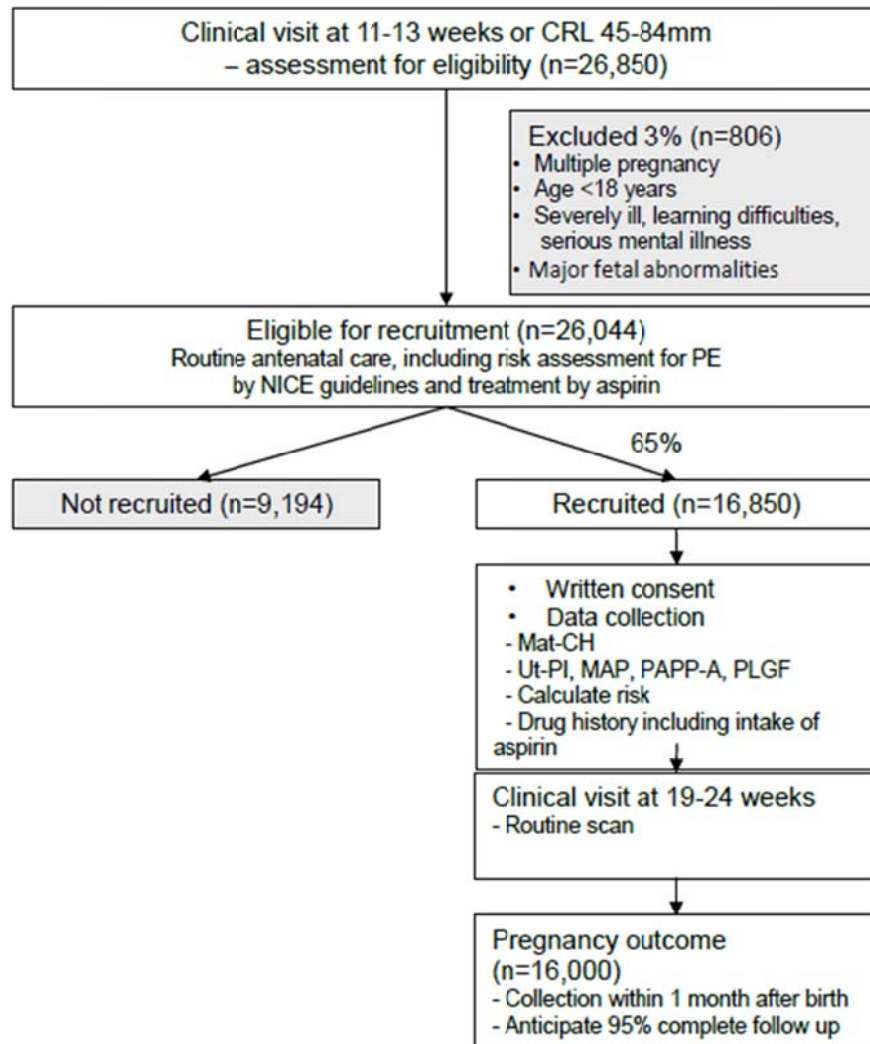
Clinical visit at 11-13 weeks or CRL 45-84mm
– assessment for eligibility (n=26,850)

Excluded 3% (n=806)
- Multiple pregnancy
- Age <18 years
- Severely ill, learning difficulties, serious mental illness
- Major fetal abnormalities

Eligible for recruitment (n=26,044)
Routine antenatal care, including risk assessment for PE
by NICE guidelines and treatment by aspirin

65%

Not recruited (n=9,194)

Recruited (n=16,850)

- Written consent
- Data collection
- Mat-CH
- Ut-PI, MAP, PAPP-A, PLGF
- Calculate risk
- Drug history including intake of aspirin

Clinical visit at 19-24 weeks
- Routine scan

Pregnancy outcome
(n=16,000)
- Collection within 1 month after birth
- Anticipate 95% complete follow up

Table 1. Comparison of NICE and proposed screening methods.

NICE	Mini-combined test	Combined test
Maternal demographics	Maternal demographics Medical history	Maternal demographics Medical history

Medical history	Mean arterial pressure	Mean arterial pressure
	Serum PAPP-A	Serum PAPP-A
		Serum PIGF
		Uterine artery pulsatility index

Key: serum placental growth factor (PIGF) and serum pregnancy associated plasma protein-A (PAPP-A)