This protocol template is intended for use in clinical studies which **are not** Clinical Trials of Investigational Medicinal Products (CTIMPs) that are Sponsored or Co-Sponsored by the University of Dundee and/or NHS Tayside.

All sections may be adapted to suit a particular trial/study.

Some sections may be added as required by the specific trial/study.

All blue/red instruction text is for guidance only and must be removed.

Trial/Study Protocol

Full study title: "Studying **P**regnancy's V**A**scular and **R**etinal **C**hanges**" SPARC**

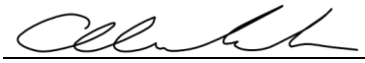
Short Title /Study Acronym	**"S**tudying **P**regnancy's V**A**scular and **R**etinal **C**hanges**" SPARC**
Sponsor	**University of Dundee**
Sponsor ID	**2-021-25**
Funder	**University of Dundee**
Chief Investigator Principal Investigator	**CI: Dr Colin Murdoch** **PI: Ms Sarah Alkhurainej (PhD student)** **2nd Academic supervisor: Prof F Khan** **3d Academic supervisor: Sarah Martins Da Silva**
IRAS Number	**347685**
Version Number and Date	**1.0** **13/3/2025**

PROTOCOL APPROVAL

Insert trial/study title: **"S**tudying **P**regnancy's V**A**scular and **R**etinal **C**hanges**" SPARC**

Signatures

The undersigned confirm that the following protocol has been agreed and approved by the Sponsor and that the Chief Investigator agrees to conduct the trial/study/study in compliance with this approved protocol and will adhere to the principles of GCP, the Sponsor SOPs, and any other applicable regulatory requirements as may be amended from time to time.

Colin Murdoch		01/02/2025
Chief Investigator	Signature	Date
Colin Murdoch		01/02/2025
Individual Responsible for Statistical Review	Signature	Date

LIST OF ABBREVIATIONS

AE	Adverse Event
CI	Chief Investigator
CNORIS	Clinical Negligence and Other Risks Indemnity Scheme
CRF	Case Report Form
DMC	Data Monitoring Committee
GCP	Good Clinical Practice
ICF	Informed Consent Form
IF	Incidental Findings
ISF	Investigator Site File
PI	Principal Investigator
REC	Research Ethics Committee
SAE	Serious Adverse Event
SOP	Standard Operating Procedures
S/TMF/SMF	Trial Master File/Study Master File
T/SMG	Trial Management Group
TSC	Trial Steering Committee

SUMMARY/SYNOPSIS

Trial/Study Title (including acronym)	**S**tudying **P**regnancy's V**A**scular and **R**etinal **C**hanges **(SPARC)**	
Trial/Study Design	Prospective imaging study	
Trial/Study Population	Pregnant women	
Sample Size	P. 44	
Planned Trial/study Period	24 months	
Clinical phase duration	During Pregnancy	
Follow up phase duration or specify none	n/a	
Primary	Objectives To investigate the potential of retinal imaging technology to assess endothelial function in women during pregnancy, in order to evaluate the effect of GH on maternal CV function.	Outcome Measures Primary Outcome Measures: 1. Retinal Vascular System 2. Endothelial Function 3. Cardiovascular Function biomarkers Secondary Outcome Measures: 1. Pregnancy-Related Health Outcomes 2. Maternal Health
Secondary	Objectives **Study – Aim:** Investigate whether retinal imaging is able to capture endothelial dysfunction in women who have complicated pregnancy. Endothelial function measured using retinal imaging and ultrasound pulse-wave Doppler and measured in correlation with serum biomarkers.	Outcome Measures 1. Primary Outcome Measures - Retinal Vascular Health measurements - Ultrasound Pulse-Wave Doppler Findings - Blood Biomarkers 2. Secondary Outcome Measures - Long-term Cardiovascular Risk Indicators
Optional Inclusion Criteria	Prospective imaging study 1- Women aged 18 or above 2- Women who are pregnant after 8 weeks 3- Women must be able to give written informed consent (ICF) Note: Women that are diagnosed with gestational hypertension (after 12 weeks) will be included. Includes the following hospital: **NINEWELLS HOSPITAL**	
Optional Exclusion Criteria	1- Maternal age less than 18 years 2- Women who are not capable of giving informed consent (ICF).	

	3- Pre-existing chronic hypertension or cardiovascular diseases diagnosed prior to the index pregnancy. 4. Women that have chronic hypertension at their booking visit (before 12 weeks) 5.Known retinal or eye disorders 6. Use of medications affecting endothelial function (e.g., ongoing anti-hypertensive therapy, anticoagulants).

1 INTRODUCTION / BACKGROUND & RATIONALE

Hypertensive disorders complicate 6-12% of pregnancies (Khedagi and Bello 2021), leading to PE and intrauterine growth restriction (Di Martino et al. 2022). PE is more commonly associated with adverse maternal and foetal outcomes such as eclampsia, stroke, renal and hepatic dysfunction, foetal growth restriction and stillbirth. According to the most recent Confidential Enquiries into Maternal Death, PE is still one of the most common causes of direct maternal deaths in United Kingdom and is also associated with increased perinatal mortality and morbidity(Shennan et al. 2012). GH is increasing in line with elevated cases of obesity and diabetes in the population (Shah et al. 2008). This is becoming more critically relevant for maternity especially as the obese and diabetic population is increasing in the younger age groups during the reproductive. Hypertensive disorders in pregnancy account for 5% (1:20) of stillbirths with no congenital malformation. Importantly, women who develop PE are also at increased long-term risk of CV disease and stroke (Honigberg et al. 2022). The impact of increased risk of CV disease later in life is under appreciated. Predictions of pregnancies at risk of hypertensive pregnancy will allow stratification of women at most risk, for future and earlier follow up. In addition, this understanding will provide insight into pathological mechanisms. An understanding of when endothelial dysfunction occurs and if it preceeds gestational hypertension will be key to developing diagnostic stratification.

Recent studies have suggested that arterial stiffness and endothelial dysfunction may be an important factor in development of GH. Similarly, myocardial impairment and chamber diastolic dysfunction was observed more frequently in GH (a severe form of GH compared with normal pregnancies. These changes in CV function may be present before the clinical onset of GH and are evident after delivery. Therefore, GH seems to be the expression of a CV "syndrome" that exists before pregnancy, become evident during pregnancy, and puts women at risk of other serious adverse events after the incident pregnancy.

Biomarkers with cardiac ultrasound at peripartum period (before delivery and within a week of giving birth) show a close connection between the endothelium indicators utilised in hypertensive pregnancy diagnosis and severity indication (Giorgione et al. 2023). Retinal imaging and subsequent AI analysis has been successfully used to assess changes in the microvascular system related to endothelial dysfunction in relation to stroke, Alzheimer's and type 2 diabetes (Cheung et al. 2021; Doney et al. 2022). However, retinal imaging has not been used extensively in hypertensive pregnancy diagnosis nor for the subsequent follow-up. Yet retinal imaging is a quick, easy and cost-effective technique to monitor endothelial health.

In our study, application of retinal imaging and subsequent analysis technology will be used to assess endothelial function during gestation and the subsequent follow up. Moreover, the application of retinal imaging and subsequent analysis technology in assessing endothelial function during gestation presents a novel approach with the potential to provide valuable insights into the microvascular changes associated with gestational endothelial dysfunction, offering a non-invasive method for monitoring maternal vascular health.

2 STUDY OBJECTIVES & OUTCOMES

To establish vascular changes in retinal and ophthalmic Doppler in normal and pregnancies associated with gestational hypertension. We hypothesise that women with GH or foetal growth restriction will have increased endothelial dysfunction, as shown by retinal and ultrasound imaging.

Table 1: Primary Objectives and Outcome Measures

Primary Objective:	**Outcome Measure:**	**Timepoint of outcome measured**
Objective: To investigate the potential of retinal imaging technology to assess endothelial function in women during pregnancy, in order to evaluate the effect of GH on maternal CV function.	- Retinal imaging - Biomarkers of Endothelial Function - Ultrasound Pulse-Wave Doppler	mild-late pregnancy for women with GH and normal pregnancies.

Table 2: Secondary Objectives and Outcome Measures

Secondary Objective:	**Outcome Measure:**	**Timepoint of outcome measured**
Study - Aim: A prospective study to image the retina in early and mid-stages of gestation and track pregnancy outcome in correlation with serum biomarkers and standard diagnostic measurement (ultrasound pulse-wave doppler) imaging.	- Retinal imaging - Biomarkers of Endothelial Function - Ultrasound Pulse-Wave Doppler	mild-late pregnancy for women with GH and normal pregnancies.

3 STUDY DESIGN

The study design is a prospective study, as it examines endothelial function in pregnant women and see how this relates to the potential development of gestational hypertension.

Study Aim: Imaging the retina in mid-stages of gestation and tracking pregnancy outcomes in correlation with serum biomarkers and for standard diagnostic measurement (ultrasound pulse-wave Doppler) imaging.

- Pregnant women will be recruited at the normal pre-natal visits (early and mid gestation scans)

- Blood samples will be taken in addition to bloods take in line with routine care (early 10-12 week scan). 20-week scan women will be requested to provide blood samples. (sFlt-1 &PLGF (clinically used to diagnose PE), endothelin-1 (marker of endothelial dysfunction))

    - Recruit women who are pregnant and follow pregnancy outcome
    - Recruit women who have gestational hypertensionGH and gestational-matched normal tensive women for CV assessment.
    - Endothelial CV assessment.
        - Retinal imaging
        - Blood sample (sFlt-1 &PLGF (clinically used to diagnose PE), endothelin-1 (marker of endothelial dysfunction))
        - Ultrasound
            - Carotid
            - Ophthalmic artery doppler

3.1 INTERVENTION

- Retinal imaging
- Blood sample (sFlt-1 &PLGF (clinically used to diagnose PE), endothelin-1 (marker of endothelial dysfunction))
- Ultrasound
    - Carotid
    - Ophthalmic artery doppler

Retinal imaging and ultrasound (including carotid and ophthalmic artery Doppler) will be conducted in the Tayside Clinical Research Centre/Clinical room at the Division of Cardiovascular Research (Blood Flow Lab~~),~~ Ninewells Hospital, by PI, Sara Alkhurainej.

Blood samples will be collected by nurses in the Clinical Research Centre (CRC). All procedures will be completed on the same day as the examination.

3.2 STUDY RECRUITMENT

Women will be recruited who are pregnant for assessment in mid and/or late term. The outcome of the pregnancy will be tracked.

Women will be invited to join the study by the care teams in clinics at Ninewells and the Tayside area

- Obstetric Antenatal Clinics (Recruitment will be overseen Dr Sarah Martins Da Silva (UoD/NHS) and Dr Ailie Grzybek (NHS))
- Assisted conception clinic (Recruitment will be overseen Dr Sarah Martins Da Silva (UoD/NHS))
- Hypertension Clinic (Recruitment will be overseen by Prof Jacob George(UoD/NHS))

This is a prospective study. Control groups will be made up of women that do not get gestational hypertension. Pregnant women that attend the Hypertension clinic may be recruited due to their gestational hypertension. Women attending the Obstetric Antenatal Clinics and Assisted conception clinics will be identified with clinical collaborators including Dr Sarah Martins Da Silva, Ailie Grzybek and Jacob George. To be able to identify potential participants from their medical records Caldicott Approval will be sought. Only with Caldicott approval will the medical records be searched.
The **care** team will approach or invite participation in the study in clinic. Any invitation will introduce the potential patient to the **study** team and will include a PIS. The care team will not follow up with patients to determine their interest in the study.
The study team will include Clinical Research Centre nurses that will assist with recruitment. The study team will confirm recruitment via a short telephone conversation prior to them attending Ninewells for the study visit.

Advertisement of the study and identification of potential participants will be identified from the NHS Tayside clinics at Ninewells Hospital including maternity clinics, ultrasound department, antenatal clinics, day assessment unit, Assisted Conception Unit, https://www.acudundee.org/ hypertension and diabetics clinics among others. Caldicott approval will be gained to allow identification of potential participants from medical records and clinical lists by the care team. Letters with PIS may be sent to the potential participants from the clinical team. We chose the following sites, since this is where pregnant women obtain their routine care.
The care team will approach or invite participation in the study either in clinic or via post/email. Any invitation will introduce the potential patient to the study team and will include a PIS will be written on behalf of the care team. The care team will not follow up with patients to determine their interest in the study. A researcher (study team) who is not in the care team can meet potential participant only with patient's permission (using a Reply slip). Or the patient contacts the study team directly using information from the PIS received from the care team
A minimum of 24 hours will be given prior to consent being obtained.
Women will be invited to join the study by the care teams in clinics at Ninewells and the Tayside area

- Assisted Conception Unit (Recruitment will be overseen Dr Sarah Martins Da Silva (UoD/NHS))
- Obstetric Antenatal Clinics (Recruitment will be overseen Dr Sarah Martins Da Silva (UoD/NHS) and Dr Ailie Grzybek (NHS))
- Hypertension Clinic (Recruitment will be overseen by Prof Jacob George(UoD/NHS))

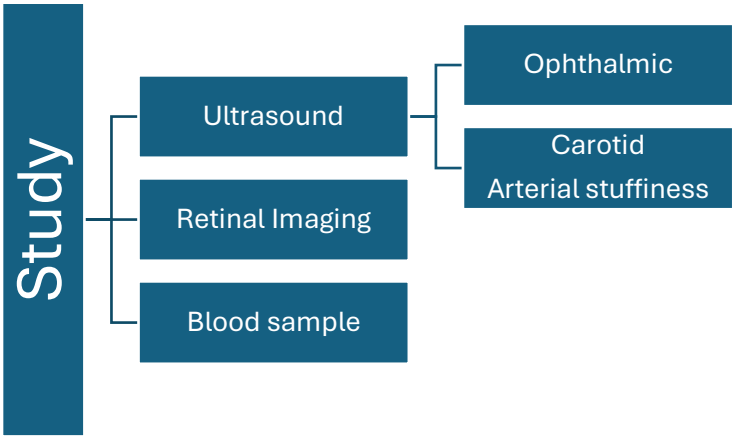
3.3 STUDY DESCRIPTION

At the commencement of the study, the care team will approach pregnant women who meet the criteria. Pregnant women will get a Participant Information

Sheet (PIS) and can reach out to the study team if they wish to participate. Each participant is only required to attend a single visit, which will last roughly 1 hour.

Pregnant volunteers will be recruited at their first (10-12 week) and second (20 weeks) scans in line with their clinical care. In addition to their clinical ultrasounds, an ophthalmic and carotid artery ultrasound will be conducted. In addition a retinal scan and blood sample taken.

3.4 STUDY FLOWCHART



3.5 STUDY MATRIX

Example Study Matrix

	Recruitment	Week 10 (+/-3wk)	Week 20 (+/-3wk)
Recruitment	V1	n/a	n/a
Cardiovascular assessment		X	X
Blood sample		X	X
Blood processing		X	X

On the same day as the blood sample, carotid artery doppler, and retinal imaging, an ophthalmic artery Doppler will be conducted. In the unlikely event that this is not possible, the ophthalmic artery doppler will be finished in 7 days.

We aim to image women up to 2 times; at 10 and 20 weeks (+/- 3 weeks) in line with their routine clinical care. However, we will accept only one visit at either of the timepoints.

STUDY ASSESSMENTS

Retinal imaging

Retinal imaging is a basic ophthalmic imaging technique which takes a photo of blood vessels at the back of the retinal. The participant needs to open her eyes and stare straight ahead at an object while the image is taken and uploaded to a computer.

Doppler Ultrasound

The ultrasound is a painless, safe test that uses sound waves to make images of the region of interest. Two regions will be examined in this study, which are Ophthalmic and Carotid region.

OPHTHALMIC ARTERY DOPPLER

The participant will be positioned in a supine position and will rest for 5 minutes. Subsequently, an ultrasound probe with sterile conduction gel will be applied to her closed upper eyelid. A quick 2D scan will be undertaken to identify anatomical features of the eye, including the optic nerve, visible as a hypoechoic band, which will be utilised as a reference. The ocular artery will be identified using colour flow, specifically in the superior and medial regions of the artery, around 15 mm behind the optic disc. Subsequently, pulsed-wave Doppler will be employed to document three to five similar waveforms.
To reduce any potential negative impact on the eyes and adhere to the ALARA ("as low as reasonably achievable") guideline, the study will be conducted in under 2 minutes per eye. Both eyes will be imaged.

CAROTID ULTRASOUND

During the test, gel is applied to the side of the neck to help the sound waves travel effectively. While lying down, a small scanner is gently placed on the skin of the neck to take the necessary pictures. This procedure is safe and may cause some pressure, but not pain, as the device moves on the neck.
The elasticity of the blood vessels will also be measured. A small pen-like device will be placed gently on the neck and at the same time a cuff is placed on the thigh (over your clothes).
The test takes about 10 minutes.

Retinal imaging and ultrasound (including carotid and ophthalmic artery Doppler) will be conducted in the Tayside Clinical Research Centre/Clinical room at the Division of Cardiovascular Research (Blood Flow Lab), Corridor L, Level 7, Ninewells Hospital, by PI Sara Alkhurainej.

Blood Samples

A 20ml blood sample will be taken from the participant during the investigation/assessment. Which are (sFlts-1, PLGF and endothelin-1). The blood sample will be collected concurrently with their usual blood tests that are clinically necessary as part of their regular clinical care.

A 20ml blood sample will be taken from the participant during the investigation/assessment by trained CRC staff for study of circulating biomarkers. This is intended to be a Prospective study and will provide data for future power analysis to estimate how many women need to be recruited.

Blood samples will be collected by nurses in the Clinical Research Centre (CRC). All procedures will be completed on the same day as the examination.

Once all the measurements have been taken, there will be no further requirements or assessments. The research specific procedure results will be recorded in the CRF only.

Blood samples from both control and case will be used to assess circulating biomarkers for example but not limited to sFlt-1 & PLGF (clinically used to diagnose preeclampsia), endothelin-1 (marker of endothelial dysfunction).

Assessment will be conducted by UoD Immunoassay Biomarker Core Facility. Blood tests for cardiometabolic profiling may also be requested (via clinical laboratory, Clinical Research Centre/NHS Tayside) or collected from patient records with prior Caldicott Approval.

Samples will be stored in Division of Cardiovascular Research, School of Medicine, Ninewells low temperature freezers (-70oC).
Patient consent will be requested to store any surplus blood for future use which will require prior ethical approval.

3.6 CONSENTING PARTICIPANTS

The consenting of participants from the groups will be performed by the CI, PI or GCP trained delegated members of the study team competent in obtaining consent for research purposes.

3.7 INELIGIBLE AND NON-RECRUITED PARTICIPANTS

The reason(s) for ineligibility will be explained to participants and any questions they have will be answered. They will be thanked for their participation in screening.

3.9 STUDY SAFETY ASSESSMENTS

Pregnant women will undergo their standard care as routine. Research bloods, ultrasound and retina imaging will be obtained at the research site following informed patient consent. This usually will occur on the visit day in the clinical room at Division of Systems Medicine, Corridor L, Level 7, Ninewells Hospital. We do not envisage that any of these will interfere with their care or are likely to cause any harm. Nevertheless, any adverse events that may potentially be related to the study will be reported to the CI who will administer appropriate clinical action.

3.10 TISSUE

A blood sample will be collected on the examination day to conduct specific tests (sFlt-1 &PLGF and endothelin-1) and, with consent, will be stored for potential future use. Samples will be stored in Division of Cardiovascular Research, School of Medicine, Ninewells low temperature freezers (-70oC).

3.11 INCIDENTAL FINDINGS

Any incidental findings (IF: previously undiagnosed condition) considered to be clinically significant will be reported to the participant's GP and/or consultant by the CI or Site PI, with the consent of the participant.

3.12 STUDY POPULATION

Our study populations are defined as follows
The study design is prospective study. All pregnant women will be included at the start except for exclusion criteria see (3.15). Outcome of pregnancy will be documented and participants will be placed into one of two groups retrospectively:

3.13 NUMBER OF PARTICIPANTS

Study: 22 PARTICIPANTS
11 participants for case group
11 participants for control group

3.14 INCLUSION CRITERIA

Case group:

Women who

- Are Aged 18 or above
- Are Able to give written informed consent (**ICF)**
- Are in mid-to-late gestation.
- Were diagnosed with Gestational hypertension (systolic BP ≥ 140 mmHg or diastolic BP ≥ 90 mmHg) in their pregnancy.
- Do not have cardiac or ophthalmic disorders

Control group:

Pregnant women that are

- Aged 18 or above
- Able to give written informed consent (**ICF)**
- Have no gestational hypertension (normal pregnancy/ non-hypertension)
- Do not have cardiac or ophthalmic disorders

3.15 EXCLUSION CRITERIA

Exclusion criteria are the same for both case and control groups:

Case group:

Women who

- Women with ophthalmic disorders
- Women who have hypertension during pregnancy
- Maternal age less than 18 years at delivery.
- Women who are not capable of giving informed consent (**ICF)**.
- Individuals participating in the clinical phase of another interventional study or have done so within the last 30 days (unless they are participating in the follow-up phase of another interventional trial/study, or who are enrolled in an observational study, will be co-enrolled where the CIs of each study agree)

Control group:

- Women with any history of gestational hypertension (systolic BP ≥ 140 mmHg or diastolic BP ≥ 90 mmHg))
- Women with ophthalmic disorders
- Women who had hypertension prior to pregnancy
- Maternal age less than 18 years at delivery.
- Women who are not capable of giving informed consent (**ICF)**.
- Individuals participating in the clinical phase of another interventional study or have done so within the last 30 days (unless they are participating in the follow-up phase of another interventional trial/study, or who are enrolled in an observational study, will be co-enrolled where the CIs of each study agree).

4 PARTICIPANT SELECTION AND ENROLMENT

For the following reasons, there is very little chance of researcher bias: 1. Objective examinations are used to measure the vascular function in pregnancy non-invasively; the majority of these tests are automated. 2. The woman's own attending clinicians will diagnose pregnancy problems, like PE, gestation hypertension and gestation diabetic mellitus based on the hospital's established criteria.

4.1 IDENTIFYING PARTICIPANTS

At the commencement of the study, the care team will approach pregnant women who meet the criteria. Patients will get a Participant Information Sheet (PIS) and can reach out to the study team if they wish to participate. Each participant is only required to attend a single visit, which will last roughly 1 hour.

- Pregnant volunteers will be recruited at their first (10-12 week) and second (20 weeks) scans in line with their clinical care. In addition to their clinical ultrasounds, an ophthalmic and carotid artery ultrasound will be conducted. In addition a retinal scan and blood sample taken.

Potential subjects will be identified by the care team in the following clinics

- Hypertension Clinic (Recruitment will be overseen by Prof Jacob George(UoD/NHS))
- IVF clinic (Recruitment will be overseen Dr Sarah Martins Da Silva (UoD/NHS))

- Obstetric Antenatal Clinics (Recruitment will be overseen Dr Sarah Martins Da Silva (UoD/NHS) and Dr Ailie Grzybek (NHS))

Advertising of the study in gyn clinics via posters adverts to invite subjects to volunteer by contacting the study team.

Potential subjects will be invited during their clinic visit and provided with a Participant Information Sheet (PIS), which includes details on how to contact the study team if they wish to participate. Additionally, interested participants will be contacted via a short telephone call or email to screen them for the inclusion/exclusion criteria.

4.2 CONSENTING PARTICIPANTS

The study team will consent the participants and ensure they have a minimum of 24h from receiving the PIS before requesting consent.
Consent Process: Consent will be obtained from each participant group prior to the start of the study. Participants will have the option to provide consent either electronically via email before the examination day or in person at the examination room immediately prior to the examination. A digital or hard record of consent will be kept securely in Cardiovascular Research Offices. During consent it will be made clear how the participants can withdraw from the study and their rights regarding the data, highlighting the information on the PIS.

Where a participant requests to speak with a physician from the study team the consent process will not be completed until the participant has spoken to the physician and had all their questions answered to their satisfaction.

The informed consent process will be conducted in compliance with TASC SOP07: Obtaining Informed Consent from Potential Participants in Clinical Research

4.3 SCREENING FOR ELIGIBILITY

The consenting of participants from the groups will be performed by the CI, PI or GCP trained delegated members of the study team competent in obtaining consent for research purposes.

4.4 INELIGIBLE AND NON-RECRUITED PARTICIPANTS

The reason(s) for ineligibility will be explained to participants and any questions they have will be answered. They will be thanked for their participation in screening.

4.5 WITHDRAWAL PROCEDURES

Participants are free to withdraw from the study at any time. If at any time the participant formally withdraws her consent for future participation and disclosure of future information, no further evaluations will be performed, and no additional data should be collected. Data collected before withdrawal will be retained and used in the study analysis, unless the participant requests

otherwise. If participants withdraw during the visit, they will have the option to request that data already collected is not to be used in the study.

5 DATA COLLECTION & MANAGEMENT

Data management will adhere to the Tayside Academic Sciences Centre Standard Operating Procedures (TASC SOPs) on Data Management, specifically focusing on Data Management Systems in Clinical Research. The data management system (DMS) will utilize EXCEL and align with the study protocol, Case Record Form (CRF), and investigators' specific needs. The CRF will gather essential information to fulfill the study objectives and ensure participant eligibility and safety. Maintaining medical confidentiality and data protection laws, the database management follows relevant guidelines. The University of Dundee acts as the Data Controller, with the Chief Investigator (CI) serving as the Data Custodian. While the CI can delegate CRF tasks, they are accountable for the completeness, accuracy, and coherence of the CRF. Any issues or questions will be addressed by the CI or a designated member of the research team.

5.1 DATA COLLECTION

The patients will be given an identification number to ensure anonymity. Data collected will include age, previous medical history, medications, diagnosis, treatment, blood report findings, and personal history. This clinical data will be used in the analysis of results and will be used to correlate with the findings of the CV assessments. The data will be collected by the research team on a paper Case Record Form (CRF), which will not hold any name, initials, and CHI, with subsequent transcription into an Excel database. Electronic storage will be in an encrypted form on a University of Dundee password-protected device.
Study assessments will be conducted by the PI and a trained research member of the team under the supervision of the CI.
On the same day as the blood sample, carotid artery doppler, and retinal imaging, an ophthalmic artery Doppler will be conducted. In the unlikely event that this is not possible, the ophthalmic artery doppler will be finished in 7 days.

5.2 DATA MANAGEMENT SYSTEM

Data management will be conducted in compliance with TASC SOPs on Data Management, Data Management Systems in Clinical Research. The data management system (DMS) will be EXCEL. The DMS will be based on the protocol and CRF for the study and individual requirements of the investigators. The CRF will collect only information that is required to meet the aims of the study and to ensure the eligibility and safety of the participant. The database is managed in line with all applicable principles of medical confidentiality and data laws. The Data Controller will be the University of Dundee and the Data Custodian will be the CI. The CI may delegate CRF completion but is responsible for completeness, plausibility, and consistency of the CRF. Any queries will be resolved by the CI or delegated member of the study team.

Security measures and other mechanisms to protect personal data	Data will be stored n University of Dundee one drive which is safe and protected by UoD-IT. Data in the paper files will be locked in a secure cabinet in the Cardiovascular Medicine division.

Only members of the study group (~4 people) will have access to the files. Caldicott approval will be sought for the principle investigator and Chief Scientist.

6 STATISTICS AND DATA ANALYSIS

Descriptive statistics will be used in this study. To compare between groups, we will use a t-test and binary logistic regression, depending on the situation. Logistic regression analysis will be conducted to evaluate and account for any confounding factors. In order to validate the non-invasive techniques, a Bland-Altman analysis will be conducted to evaluate the comparability of the methods. Regression analysis to identify predictors of pregnancy outcomes. The Statistical analysis will be performed in SPSS (SPSS Statistics. 2022. Version 29.0.0.0. International Business Machines Corporation, New York, United States).

6.1 SAMPLE SIZE CALCULATION

Comparison of CV function in women at high and low risk of PE

Ninewells has approximately 4500 births per year with approximately 450 cases of gestational hypertension. Our goal here is to describe the evolution of maternal microvascular function in women that develop gestational hypertension comparing to those that do not develop gestational hypertension.

Our previously published studies (Khan et al 2005 Hypertension, https://doi.org/10.1161/01.HYP.0000186328.90667.95; Changes in Endothelial function preceded the clinical disease in women in whom PE develops) demonstrated changes in microvasculature at 26 weeks in n=15 women with PE compared to normotensive (n=54). Using this previous data, a Power Analysis (G*power)) -A Piori carried out to compute the required sample size for the Wilcoxon-Mann Whitney test (2 groups). With

$\alpha\alpha$

= 0.05 and power of 95% to detect an effect size of 25%, each group requires a n=11.

As this is a prospective study to obtain measurements at the onset of gestational hypertension, we predict we need to recruit 110 women to obtain the 10% of women that develop gestational hypertension. Women who are high-risk or that have been newly diagnosed, i.e. attending hypertensive clinics, will be recruited

Further details:

- During-Pregnancy: Recruit 110 women to account for the 10% who will develop gestational hypertension.

Sample size required for each group (high vs. low risk):11 women per group

- Total Participants:

-During-Pregnancy: 110 women (recruitment to achieve the final sample of 22 women)

6.2 PROPOSED ANALYSES

**Study
- **Aim**: To investigate correlations between retinal imaging, ultrasound and blood sample biomarkers and future CVD risk factors in mid-late stages of gestation.
- Hypothesis:
Cardiovascular Assessment:
Blood biomarker (sFlt-1, PlGF)) will correlate with retinal vascular changes and Dopple rultrasound indices, potentially predicting future CVD.
- **Methods**:
- Perform *correlational analysis* between retinal metrics and blood biomarkers to examine associations.
- Conduct *logistic regression analyses* to assess whether retinal imaging metrics in early pregnancy predict future CVD risk.
- *Multivariable regression modeling* to control for confounding factors such as maternal age, BMI, and pre-existing health conditions.

Data Protection Impact Assessment (DPIA)
- We aim to understand the link between retinal imaging biomarkers and cardiovascular function in gestational hypertension, predicting CVD risk factors and informing early interventions.
What will be the effect on data subjects?
-Positive Impact**: Early detection of risk factors for cardiovascular health, potentially improving maternal health management and long-term care.
What is the nature of the University's relationship with the data subjects?
- The University serves as the primary researcher and data controller, conducting the study with informed consent from participants. The relationship is one of informed, voluntary participation in medical research.
Are they expecting you to use their data in the way you've proposed?
- Yes, participants are fully informed through the consent process about the study's objectives, data collection, and how their data will be used for research on maternal and cardiovascular health.
Are there prior concerns with this proposed activity in the public domain that you are aware of?**
- Retinal imaging and biomarker studies in maternal health are generally well-accepted, though concerns about the use of sensitive medical data highlight the importance of rigorous data protection.
Is the University signed up to any codes of conduct in respect of the data processing?
- The University adheres to all GDPR guidelines and maintains compliance with institutional data protection policies, ensuring ethical handling and storage of sensitive health data.
Are there sufficient resources to meet the data security requirements for this type of processing?

- Yes, the University has sufficient resources to meet data security needs, including secure data storage, access control, and compliance checks to protect participants' data throughout the study.

6.3 MISSING DATA

Data entry and checking will be undertaken by a single entry with a second look. The data entry and checking process will be decided according to risk. Data that is recorded in the CRF that is not source document in itself will be consistent with the source documents or the discrepancies explained. Checks will be made on all missing values and values out with normal or expected ranges and that values entered are of the correct type: i.e. numerical instead of text. Logical checks will be performed to ensure consistent reporting between relevant fields and that there are no differences between fields. Data checking will continue until all missing data and/or inconsistent values have been corrected or clarified. When data checking is complete, with no outstanding data queries, the database will be locked, using the Protect Worksheet function of EXCEL, as FINAL RESULTS. The EXCEL spreadsheet will remain archived on the Dundee University secure server for 5 years for the retention of essential documents, thereafter it will be deleted.

6.4 TRANSFER OF DATA

Study data transcribed onto the paper CRFs will be transported from the site of the study visit, to the place of analysis and storage (University of Dundee), in a lockable case. Data transfer will follow the standard University of Dundee guidelines.

7 STUDY MANAGEMENT AND OVERSIGHT ARRANGEMENTS

7.1 STUDY MANAGEMENT GROUP

The study, no matter the number of participants or number of collaborating sites, should establish a study management group (T/SMG) to set and review the day to day management of the study. The T/SMG should include the CI, PIs and co-applicant.

A Delegation Log must be in place at each site.

The study will be co-ordinated by a Study Management Group (SMG), consisting of e.g. the grant holder Chief Investigator (CI), Principal Investigators (PI) (Sarah Alkhurainej).

7.2 STUDY STEERING COMMITTEE

No SC will be established, the remit will be carried out as part of the SMG.

7.3 DATA MONITORING COMMITTEE

No DMC will be established, the data monitoring for this study will be conducted as the remit as part of the SMG. Further data monitoring will be performed by University of Dundee Thesis monitoring committee and PhD exam for the principle investigator (Sarah Alkhurainej)

7.4 INSPECTION OF RECORDS

The CI, PIs and all institutions involved in the study will permit study related monitoring, audits, and REC review. The CI agrees to allow the Sponsor or, representatives of the Sponsor, direct access to all study records and source documentation.

8 GOOD CLINICAL PRACTICE

8.1 ETHICAL CONDUCT OF THE STUDY

The study will be conducted in accordance with the principles of good clinical practice (GCP).

In addition to Sponsorship approval, a favourable ethical opinion will be obtained from the appropriate REC and appropriate NHS R&D approval(s) will be obtained prior to commencement of the study.

8.2 CONFIDENTIALITY AND DATA PROTECTION

The CI and trial staff will comply with all applicable medical confidentiality and data protection principles and laws with regard to the collection, storage, processing and disclosure of personal data.

The CI and trial staff will also adhere to the NHS Scotland Code of Practice on Protecting Participant Confidentiality or equivalent.

All trial records and personal data will be managed in a manner designed to maintain participant confidentiality. All records, electronic or paper, will be kept in a secure storage area with access limited to appropriate trial staff only. Computers used to collate personal data will have limited access measures via user names and passwords.

Personal data concerning health will not be released except as necessary for research purposes including monitoring and auditing by the Sponsor, its designee or regulatory authorities providing that suitable and specific measures to safeguard the rights and interests of participants are in place.

The CI and trial staff will not disclose or use for any purpose other than performance of the trial, any personal data, record, or other unpublished, confidential information disclosed by those individuals for the purpose of the trial. Prior written agreement from the Sponsor will be required for the disclosure of any said confidential information to other parties.

Access to collated personal data relating to participants will be restricted to the CI and appropriate delegated trial staff.

Where personal data requires to be transferred, an appropriate Data Transfer Agreement will be put in place.

Published results will not contain any personal data that could allow identification of individual participants.

The University of Dundee has a code of conduct and guidelines for data protection. The investigators all receive regular training in data protection measures. The University provides secure digital environment (Microsoft 365). There are data protection officers in TASC, University of Dundee who the research team can consult. The responsibility lies with all of the research team.

There are procedures in place if there is a data breach and these will be followed.

8.3 INSURANCE AND INDEMNITY

The University of Dundee is Sponsoring the study.
Insurance – The University of Dundee holds Clinical Trials indemnity cover which covers the University's legal liability for harm caused to patients/participants..
Where the study involves University of Dundee staff undertaking clinical research on NHS patients, such staff will hold honorary contracts with Tayside Health Board which means they will have cover under Tayside's membership of the CNORIS scheme.
Indemnity The Sponsor does not provide study participants with indemnity in relation to participation in the Study but has insurance for legal liability as described above.

9 ADVERSE EVENTS

Adverse Event (AE)	Any untoward medical occurrence in a clinical research participant which does not necessarily have a causal relationship with study participation
Serious Adverse Event (SAE)	A serious adverse event is any untoward medical occurrence that: • results in death • is life threatening • requires hospitalisation or prolongation of existing hospitalisation • results in persistent or significant disability or incapacity • is a congenital anomaly or birth defect • Or is otherwise considered serious

9.1 DEFINITIONS

The following definitions will be used in this study, based on the American College of Obstetrics and Gynaecology (ACOG, 2002).

1- Chronic hypertension: BP ≥140/±90 mm Hg before pregnancy or before the 20th week of gestation

2- Preeclampsia-eclampsia: A pregnancy-specific disorder that is a multisystem disease characterized by hypertension ≥140/±90 mm Hg on ≥2 occasions at least 6 hours apart, and proteinuria ≥300 mg in a

24-hour urine collection, after 20 weeks' gestation. The convulsive form of PE is eclampsia and affects 0.1% of all pregnancies.

3- Preeclampsia superimposed on chronic hypertension: Up to 30% of women with chronic hypertension develop PE, as heralded by the occurrence of de novo proteinuria in the third trimester. In women with chronic hypertension and preexisting proteinuria (ie, before 20 weeks of gestation), the diagnosis of superimposed PE is likely with any of the following findings: sudden increase in proteinuria, sudden worsening of previously well-controlled BP, new-onset thrombocytopenia, or elevated liver function tests.

4- Gestational hypertension: New onset of hypertension ≥140/±90 mm Hg on ≥2 occasions at least 6 hours apart, after 20 weeks' gestation, in the absence of proteinuria, <300 mg in a 24-hour urine collection. If BP returns to normal by 12 weeks postpartum, the diagnosis of transient hypertension of pregnancy can be assigned. If elevated BP persists, the diagnosis of chronic hypertension is made.

Other conditions:

Pre-gestational diabetes
- Type I – insulin-requiring diabetes
  - Participant taking (or prescribed) insulin prior to pregnancy
- Type II – non-insulin requiring diabetes
  - Participant taking (or prescribed) oral diabetic agents prior to pregnancy

Gestational diabetes
- Prior pregnancy affected by gestational diabetes
  - No interval diagnosis of diabetes
- Current pregnancy affected by gestational diabetes

9.2 RECORDING AND REPORTING AE

All SAEs will be recorded on the SAE Log in the CRF and will be assessed for severity by the CI or delegate. SAEs will be recorded from the time a participant consents to join the study until the participant's last study visit. SAEs will be coded using MedRA

The Investigator will make a clinical judgment as to whether or not an AE is of sufficient severity to require the participant's removal from the study. A participant may also voluntarily withdraw from treatment due to what he or she perceives as an intolerable AE. If either of these occurs, the participant should, if required, be offered an end of study assessment and be given appropriate care under medical supervision until symptoms cease, or the condition becomes stable. SAEs will be followed up until 30 days after participant's last visit.

The CI or delegate will ask about the occurrence of SAEs and hospitalisations at every visit during the study. **SAEs which are both unexpected and related**

to study participation will be submitted on an HRA NCTIMP Safety Report form to the REC by the CI, within 15 days of becoming aware of the SAE, and copied to the Sponsor Research Governance Office.

Worsening of the condition under study will not be classed as an AE, but will be defined as an outcome. Pre-specified outcome(s) will not be classed as an AE but as an outcome. Elective admissions and hospitalisations for treatment planned prior to randomisation, where appropriate, will not be considered as an AE. However /SAEs occurring during such hospitalisations will be recorded.

10 ANNUAL REPORTING REQUIREMENTS

Annual reporting will be conducted in compliance with TASC SOP 15: Preparing and Submitting Progress and Safety Reports in CTIMPs and Non-CTIMPs, as a condition of sponsorship and as a condition of a favourable opinion from a REC. An HRA Annual Progress Report for NCTIMPs will be prepared and submitted by the CI to REC, and copied to the Sponsor, on the anniversary date of the REC favourable opinion.

Any safety reports additional to SAE reports, for example, reports of a DMC, will be sent by the CI to REC, with a Safety Report Form, and to the Sponsor.

11 STUDY CONDUCT RESPONSIBILITIES

11.1 PROTOCOL AMENDMENTS, DEVIATIONS AND BREACHES

The CI will seek approval for any amendments to the Protocol or other study documents from the Sponsor, REC and NHS R&D Office(s). Amendments to the protocol or other study docs will not be implemented without these approvals.

In the event that a CI needs to deviate from the protocol, the nature of and reasons for the deviation will be recorded in the CRF, documented and submitted to the Sponsor as a potential breach report. If this necessitates a subsequent protocol amendment, this will be submitted to the Sponsor for approval and then to the appropriate REC and lead NHS R&D Office for review and approval.

In the event that a serious breach of GCP or protocol is suspected, this will be reported to the Sponsor Governance Office immediately

Research team will follow University of Dundee Guidelines and will work closely with TASC and NHS Tayside informing all of the data officers of any potential incident. The patients/participants will be informed following the guidelines set out by University of Dundee/TASC/NHS Tayside. Preventative measures will be agreed by the appopriate data officers.

11.2 STUDY RECORD RETENTION

No NHS medical case notes will be retained. Archiving of study documents will be for five years after the end of study or in the case of anonymised data will be retained for 15 years.

We will only be collecting data that is specifically required for the project. We will be collecting data from ~22 participants.

Data will be anonymised and retained for 15 years, in requirement of publishing results from the study.
University guidelines and IT department will be provide the adequate procedures to securely delete personal data. Health Informatics Centre will provide advice and support to anonyomize the data and provide the means to securely retain the code for a limited period (5 years).

11.3 END OF STUDY

The end of study is defined as last patient visit and database lock. The Sponsor, CI and/or the SC have the right at any time to terminate the study for clinical or administrative reasons.

The end of the study will be reported to the Sponsor and REC within 90 days, or 15 days if the study is terminated prematurely. The CI will ensure that any appropriate follow up is arranged for all participants.

A summary report of the study will be provided to the Sponsor and REC within 1 year of the end of the study.

12 REPORTING, PUBLICATIONS AND NOTIFICATION OF RESULTS

12.1 AUTHORSHIP POLICY

Ownership of the data arising from this study resides with the study team and their respective employers. On completion of the study, the study data will be analysed and tabulated, and a clinical study report will be prepared.

12.2 PUBLICATION

The clinical study report will be used for publication and presentation at scientific meetings. Investigators have the right to publish orally or in writing the results of the study.

Summaries of results will also be made available to Investigators for dissemination within their clinical areas (where appropriate and according to their discretion).

12.3 PEER REVIEW

The protocol will undergo peer review through referees of the journal to which the paper (and its protocol) will be submitted, ensuring the quality and credibility of the research. Sponsors have also performed a peer review in accepting the project for PhD study.

13 REFERENCES

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