Trial/Study Protocol

Full study title: Tracking Retinal and Cardiovascular Effects in pregnancies with gestational hypertension (TRACE): Assessments post-pregnancy.

Short Title /Study Acronym	**TRACE**
Sponsor	**University of Dundee**
Sponsor ID	**2-003-25**
Funder	**University of Dundee**
Chief Investigator Principal Investigator	**CI: Dr Colin Murdoch** **PI: Ms Sarah Alkhurainej (PhD student)**
IRAS Number	**350550**
Version Number and Date	**1.0** **13/3/2025**

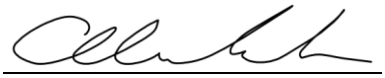
PROTOCOL APPROVAL

Insert trial/study title

Tracking Retinal and Cardiovascular Effects in pregnancies with gestational hypertension (TRACE): Assessments post-pregnancy.

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		13/03/2025
Chief Investigator	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)	Tracking Retinal and Cardiovascular Effects in pregnancies with gestational hypertension (TRACE): Assessments post-pregnancy.	
Trial/Study Design	Prospective imaging study	
Trial/Study Population	Pregnant women	
Sample Size	Post P. 22	
Planned Trial/study Period	24 months	
Clinical phase duration	Post Pregnancy: 6 – 60 months after pregnancy	
Follow up phase duration	n/a	
Primary	Objectives To investigate the potential of retinal imaging technology to assess endothelial function in women post-pregnancy, in order to evaluate the effect of GH on maternal CV function.	Outcome Measures Primary Outcome Measures: 1. Retinal Vascular System of measurement 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 had a previous complicated pregnancy (0.5-5years). 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	**Study :** **Inclusion Criteria for case group** 1- Women aged 18 or above 2- Women who had a previous pregnancy complication (e.g., gestational hypertension, preeclampsia) 6 months to 5 years postpartum. 3- Women must be able to give written informed consent (ICF) Control group: 1- Women aged 18 or above 2- Women who had a previous normal pregnancy, 6 months to 5 years postpartum. 3- Women must be able to give written informed consent (ICF) Includes the following hospital: **NINEWELLS HOSPITAL**	

Optional Exclusion Criteria	**Exclusion Criteria for case group** 1- Maternal age less than 18 years at booking. 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. 2. Current pregnancy or recent pregnancy (within the past 6 months). 4. Known retinal or eye disorders 5. Use of medications affecting endothelial function (e.g., ongoing anti-hypertensive therapy, anticoagulants). **Exclusion Criteria for control group ** 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. 2. Current pregnancy or recent pregnancy (within the past 6 months). 4. Known retinal or eye disorders 5. 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 the endothelial dysfunction and if this can be imagined in women with GH either during pregnancy or some time afterwards 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 PE (a severe form of GH compared with normal pregnancies. These changes in CV function may be present before the clinical onset of PE and are evident after delivery. Therefore, PE 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 TRIAL/STUDY OBJECTIVES & OUTCOMES

The overarching aim of this project proposal is to understand if retinal imaging and Ophthalmic doppler can be used as a biomarker for GH. 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**
To assess if women who had gestational hypertension have long-term CV dysfunction.	- *Retinal imaging* - *Ultrasound Pulse-Wave Doppler*	***Post-Pregnancy**:* *6 to 60 months postpartum for women with previous GH and normal pregnancies.*

Table 2: Secondary Objectives and Outcome Measures

Secondary Objective:	**Outcome Measure:**	**Timepoint of outcome measured**
Investigate whether retinal imaging correlates with ultrasound pulse-wave Doppler and serum biomarkers in women who have endothelial dysfunction.	- *Retinal imaging* - *Biomarkers of Endothelial Function* - *Ultrasound Pulse-Wave Doppler*	***Post-Pregnancy**:* *6 to 60 months postpartum for women with previous GH and normal pregnancies.*

3 STUDY DESIGN

The study design is a cross-sectional, as it examines past cases of women who had complicated pregnancies and measures outcomes related to endothelial dysfunction.

Aim: Investigate whether retinal imaging and ophthalmic pulse wave doppler can capture underlying endothelial dysfunction in women who had a previous complicated pregnancy (0.5-5 years). Endothelial function measured using retinal imaging and ultrasound pulse-wave Doppler (Year 0.5-5).

- Recruit women who had GH and gestational-matched normal tensive women for CV assessment. between 6 and 60 months
- 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 Preeclampsia), 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), Corridor L, Level 7, 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 into either the case or control group. Inclusion/Exclusion Criteria are detailed below on page 12.

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

- 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))

The Health Informatic Centre (UoD) and SHARE will use Electronic Health Records and the SHARE database to identify women for the control group that have consented to be contacted. Correspondence (letter, email) will be used to request women to join the study.

Control group, women attending the Obstetric Antenatal Clinics and IVF 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.

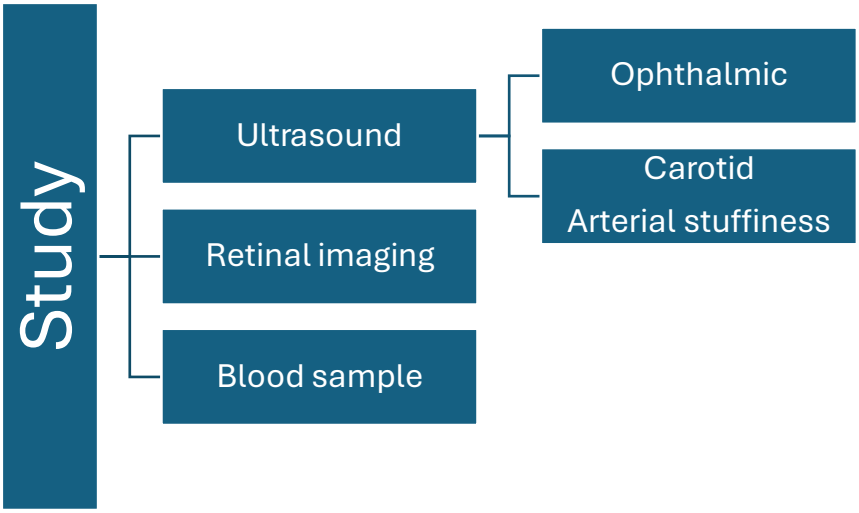
Further Post-natal clinics in Ninewells and Tayside area will be requested to display posters or circulate emails to volunteer for recruitment. (First contact with Sarah Alkhurainej, and recruitment will be assisted by Clinical Research Centre).

The **care** team will approach or invite participation in the study either in clinic or via post. 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.

Each participant is only required to attend a single visit, which will last roughly 1 hour

3.3 STUDY FLOWCHART



3.4 TRIAL/STUDY MATRIX

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.

3.5 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. The PI is fully trained in this procedure.

Blood Samples

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 cross-sectional 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), centrifuge to separate plasma from serum using standard procedures will be conducted for the control group of participants.

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 which will be registered with Tayside Biorepository or destroyed. Future use which will require prior ethical approval from Tayside Biorepository.

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.

3.8 STUDY SAFETY ASSESSMENTS

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.

3.9 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).

3.10 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.

3.11 STUDY POPULATION

Our study populations are defined as follows:

- **Case Group:** Women who experienced gestational hypertension.
- **Control Group:** Women who experienced non-hypertensive pregnancies.

Both will be evaluated 6-60 months postpartum.

3.12 NUMBER OF PARTICIPANTS

Study: 44 PARTICIPANTS
22 participants for case group
22 participants for control group

3.13 INCLUSION CRITERIA

Case group:

Women who

- Are Aged 18 or above
- Are Able to give written informed consent (**ICF)**
- Have given birth in the last 6-60 months.
- Were diagnosed with Gestational hypertension (systolic BP ≥ 140 mmHg or diastolic BP ≥ 90 mmHg) in their last pregnancy.

Control group:

Women that are

- Aged 18 or above
- Able to give written informed consent (**ICF)**
- Have no history of gestational hypertension

3.14 EXCLUSION CRITERIA

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

Case group:

Women who

- 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 trial/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 trial/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 post 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

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))

Caldicott approval will be requested to allow the care team to use clinical records to identify potential subjects. Letters or emails will be sent from the care team. Or the potential subjects will be invited when they attend the clinic and provided a Participant Information Sheet (PIS) with details to contact the study team if they wish to participate.

Also Health Informatic Centre will help identify potential subjects using the SHARE database where people have consented to be contacted.

Advertising of the study in post-natal clinics, GP surgeries via posters or email adverts to invite subjects to volunteer by contacting the study team.

Potential subjects will be sent a Participant Information Sheet (PIS). A short telephone call or email will be used to screen the participants for the inclusion/exclusion criteria.

Each participant is only required to attend a single visit, which will last roughly 1 hour.

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.

For adults who lose capacity their previous wishes will remain legally binding and this will remain valid unless the protocol changes significantly. If this occurs and further consent is required from a participant who has lost capacity, the appropriate person will be asked for their consent. In all cases the CI or delegate will consult with carers and take note of any signs of objection or distress from the participant – the participant will be withdrawn if they raise objection. Where appropriate the participant will be withdrawn from any further clinical intervention and agreement will be sought from a carer to allow data collection.

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

4.3 SCREENING FOR ELIGIBILITY

The study team will screen for eligibility of potential subjects in a telephone or email exchange prior to consenting. This will be performed by the study team 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.

5 DATA COLLECTION & MANAGEMENT

Data management will adhere to the Tayside Academic Sciences Centre Standard Operating Procedures (SPONSOR 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 data will be collected by the CI or delegated members of the research team, saved onto the University of Dundee (UoD) computer's hard drive and routinely backed up onto another storage medium. Relevant data from the CRF and computerised tests will be transcribed into an electronic database. Results will be recorded onto the Case Record Form (CRF), kept securely on password-protected university computer servers by study collaborators at the University of Dundee.

- **Data Collection**
- **Types of Personal Data Collected:**
    - Personal data: Information directly provided by participants (questionnaires).
    - Health data: Clinical measurements from devices (ultrasound), maternity records, and clinical portals.
    - Outcome-related data: Accessed via Clinical Portal (approved through Caldicott Guardian).
    - Data will be coded and anonymized before analysis by the PI (Sara Alkhurainej) who will gain Caldicott Approval. The clinical PI (Dr Sarah Martin Da Silva) will oversee the anonymisation of clinical data
    - The researcher and CI will be responsible for data analysis a. If statistical support is needed will be gained from UoD Statistical support. Data Analysis group or DEBU.
- **Methods of Data Collection:**
    - **Direct Collection:** Participants will provide data via questionnaires.

- **Clinical Systems:** Additional data will be accessed from Clinical Portal and Maternity Records.
- **Devices:** Ultrasound measurements will be recorded on ~~patient sheets.Case~~ Record Form (CRF)

- **Who Will Collect the Data & Under What Conditions?**
  - Data from clinical records will be collected by the PI (PhD Student) and overseen by the CI
  - Clinical data will only be accessed with appropriate approvals (e.g., Caldicott approval for Clinical Portal access).
  - Following UK Data Service guidelines, direct identifiers (suca as name, DOB, address) will not be held in conjunction with quantitative data.
  - However, name and address will be held separately from quantitative data on consenting forms to allow contact with patients such as providing final project results.
  - Experimental data will be collected by the study team (PI- PhD student) during the assessment.

- **Data Use, Storage, and Retention**
- **How Will Data Be Collected, Used, Stored, and Deleted?**
  - Data will be recorded on paper forms and then transferred to a secure Excel document.
  - The Excel document will be stored on University of Dundee's secure computer system with password protection.
  - Identifiable data will be removed, and a coding system will be used for analysis.
  - Only authorized personnel will have access to the data.
- **Data Retention & Deletion:**
  - Data will be collected for **12-18 months**.
  - All data will be securely stored for **15 years**, after which it will be permanently deleted using secure deletion methods. CI (academic supervisor) is responsible for carrying out this task.
  -
- **How Much Data Will Be Collected & Frequency?**
  - Data from **approximately 44 participants**.
  - Data collection will be done periodically over the **12-18 month** project duration.

- **Data Flow & Security Measures**
- **Data Flow:**
  - Data is first collected on paper forms and ultrasound records.
  - Transferred to an Excel document stored on a **University of Dundee secure system**.
  - Clinical outcome data will be accessed through Clinical Portal and linked to study data (without identifiers).

- **Technology & Security Controls:**
  - **Storage:** Data will be stored on **University of Dundee secure computers** (password-protected).
  - **Cloud Storage:** Secure OneDrive for data backup.

  - **Encryption & Anonymization:** Personal identifiers e.g. name, address, DOB will be unlinked from quantitative data.
  - Anonymisation will be achieved by all patients given a randomly assigned patient study number. The patient study number will be used in all the quantitative analysis.
  - The deanonymized list will be held by the CI with patient information kept securely and separately from the quantitative data.
  - Data Analytics Tools: Software for analysing data (R, Prism and SPSS).
  - **Access Control:** Only authorized researchers will have access.
- **Processing of Special Category Data**
  - Health-related data, including **pregnancy outcomes and ultrasound measurements**, falls under special category data.
  - Data will be handled in compliance with GDPR and ethical guidelines, ensuring **secure storage, access restrictions, and anonymization** before analysis. Quantitative data will be anonymised by removing any direct identifiers.
  - A study specific DPIA approved by NHS IG is in place.

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 SPONSOR SOPs on Data Management, including SPONSOR SOP Data Management Systems in Clinical Research.
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 study database will be compliant with SPONSOR SOP Data Management Systems in Clinical Research.
EXCEL will be used for data management, in accordance with Sponsor SOPs. 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 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 trial/study team.

Database lock will be conducted in compliance with SPONSOR SOP Locking Clinical Study Databases.

See DPIA form.

Security measures and other mechanisms to protect personal data	Data will be stored on 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 CI.

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 SE SIZE CALCULATION

Cardiovascular (CV) changes in post-pregnancy

Our previously published study (Giogione et al 2023; Ultrasound in Obs&Gyn; Angiogenic markers and maternal echocardiographic indices in women with hypertensive disorders of pregnancy. **https://doi.org/10.1002/uog.27474**) demonstrated changes in CV by ultrasound in women 6-18months post pregnancy with n=25 control comparing n=34 gestational hypertension women. Using this previous data, a Power Analysis (G*power) Piori carried out to compute the required sample size for the Wilcoxon-Mann Whitney test (2 groups). With α= 0.05 and power of 95% to detect an effect size of 20%, each group requires a size of=22.

Further details:

- A total of 44 women are required (both groups combined):

Case group: n= 22

Control group: n=22

6.2 PROPOSED ANALYSES

To determine if retinal imaging can capture signs of endothelial dysfunction in postpartum women with prior complicated pregnancies (gestational hypertension).
Methods:
Data will be analysed using widely available statistical analysis software such as SPSS. Analytical methods will include t-tests, chi-square, ANOVA, correlation and regression as appropriate.

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. 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 15 years for the retention of essential documents, thereafter it will be deleted.

7 TRANSFER OF DATA

All data will be aquired at Ninewells Hospital and Medicical School.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.

8 TRIAL/STUDY MANAGEMENT AND OVERSIGHT ARRANGEMENTS

8.1 TRIAL/STUDY MANAGEMENT GROUP

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)

8.2 TRIAL/STUDY STEERING COMMITTEE

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

8.3 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)

8.4 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.

9 GOOD CLINICAL PRACTICE

9.1 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.

9.2 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).

9.3 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.

10 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

10.1 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

10.2 RECORDING AND REPORTING AE

All adverse events will be recorded on a log that will be reviewed by CI/or medical delegate to ensure appropriate clinical care of participant to ensure appropriate clinical care of participant.. The Sponsor will be notified of all SAEs.

11 ANNUAL REPORTING REQUIREMENTS

Annual reporting will be conducted in compliance with SPONSOR 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, will be sent by the CI to REC, with a Safety Report Form, and to the Sponsor.

12 STUDY CONDUCT RESPONSIBILITIES

12.1 PROTOCOL AMENDMENTS, DEVIATIONS AND BREACHES

The CI will seek approval for any amendments to the Protocol or other study documents from the Sponsor following SOP. The sponsor SOP will be followed for any breaches.

12.2 STUDY RECORD RETENTION

Data will be retained for 15 years.

12.3 END OF STUDY

The end of study is defined database lock.

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 report of the study will be provided to the Sponsor and REC and R&D within 1 year of the end of the study.

13 REPORTING, PUBLICATIONS AND NOTIFICATION OF RESULTS

13.1 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.

13.2 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).

13.3 PEER REVIEW

The protocol will undergo peer review through referees of the journal to which the manuscript (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.

14 REFERENCES

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