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PlacEntal Acute atherosis RefLecting Subclinical systemic atherosclerosis in women up to 20 years after pre-eclampsia (PEARLS): research protocol for a cohort study

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ABSTRACT

Introduction Despite being a leading cause of female morbidity and mortality, female-specific cardiovascular disease (CVD) is understudied, underdiagnosed and undertreated. Pregnancy complications involving the placenta, including pre-eclampsia, pregnancy-induced hypertension and foetal growth restriction, are thought to reflect global maternal vascular derangements that indicate a twofold to eightfold increased risk of future CVD. This calls for a better understanding of female cardiovascular pathophysiology to allow development of targeted screening and prevention strategies.
Acute atherosis is a placental vascular lesion, which histologically resembles systemic atherosclerosis.
The PlacEntal Acute atherosis RefLecting Subclinical atherosclerosis study investigates the association between placental acute atherosis lesions and subclinical systemic atherosclerosis up to 20 years postpartum.
This study will improve our understanding of the relationship between pregnancy complications and CVD to identify potential prevention targets and treatments. In addition, it could determine whether the placenta can improve identification of young women at high risk of CVD. These women could benefit from risk-reducing interventions.

Methods and analysis This longitudinal prospective cohort study will include women who are either currently pregnant or from a historical cohort. Both groups will have placental histopathology and a single postpartum CVD assessment. The CVD assessment will include medical history taking, blood tests, electrocardiography and echocardiography. Additionally, coronary CT angiography focusing on the presence of atherosclerotic plaques and calcium score will be carried out.
The currently pregnant women will either have a pre-eclamptic pregnancy (pre-eclamptic group) or an uncomplicated normotensive pregnancy (uncomplicated group), and their placenta will be collected prospectively. The single CVD assessment will be carried out 6–36 months postpartum.
Women from the historical cohort had a pre-eclamptic pregnancy 10–20 years ago. Placental tissue is available for reanalysis. The single CVD assessment will take place immediately and corresponds with 10–20 years postpartum.
Exclusion criteria are contraindications to diagnostic assessment necessities: iodinated contrast, beta-blockers or glyceryl trinitrate. Women with uncomplicated pregnancies will be excluded if they have a pre-existing auto-immune condition, chronic hypertension or diabetes mellitus. In the pre-eclamptic group, there are no additional exclusion criteria.

Ethics and dissemination Ethical approval was granted by the Medical Ethics Committee in Maastricht University

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ The prospective nature of this study and limited exclusion criteria will enable a representative population to take part, strengthening the generalisability of our findings.
⇒ Predominantly Caucasian participants are likely to be recruited in the Netherlands, which may limit our generalisability of study results to non-Caucasian ethnicities.
⇒ The use of the latest CT technology in this study will enable easier detection of subtle coronary arterial wall changes.
⇒ This study does not include an antenatal assessment of prior atherosclerosis, which means that further research is required to determine causation if a link is found between the placental acute atherosis and systemic atherosclerosis.
⇒ A degree of recruitment bias may result as participants are mainly recruited from a hospitalised population, which may lead to the inclusion of less healthy control participants compared with the general population.

Medical Centre+ (NL52556.068.15/METC152019). Participants will give written informed consent. Results will be shared in peer-reviewed journals and conference presentations.

Trial registration number NCT05500989; ClinicalTrials.gov Identifier.

INTRODUCTION

Background

Cardiovascular disease (CVD) as a leading cause of morbidity and mortality in women remains 'understudied, underdiagnosed and undertreated'.[1] A 2022 American Heart Association Call for Action[2] identified underrepresentation of women in clinical and population-based research as a primary obstacle to improving female CVD health as this hinders the effective translation of emerging evidence to the female population. It also impedes our ability to leverage female-specific risk factors to improve cardiovascular health in women.[2] Coronary artery disease (CAD) is the main constituent of CVD in women.[3] CAD is a disorder that gradually progresses from asymptomatic plaque formation to ischaemic heart disease or ischaemic cerebral disease.[4] Early intervention to slow or even halt progression before overt clinical disease develops depends on timely diagnosis. Women tend to have more diffuse, non-obstructive atherosclerotic plaque patterns compared with men, which alters the type of symptoms displayed and the recognisability of plaques on imaging.[5] This inadvertently leads to female CAD diagnoses being missed or being made at a much later stage when prevention strategies are no longer effective.[5] For expedient screening and prevention strategies in women, it is essential to understand the effect and significance of both conventional and female-specific risk factors, in relation to CAD and general CVD.

Female-specific risk factors for CVD include menopause, polycystic ovarian syndrome and pre-eclampsia.[6] Pre-eclampsia is a common pregnancy complication with a reported incidence of two to eight per cent. It is known to raise future CVD risk two to fourfold and increases incidence of atherosclerotic events by 50%.[7–9] Both European and US guidelines therefore recommend consideration of obstetric history when evaluating CVD risk.[10 11]

The placenta, often discarded after delivery, has the potential to provide a non-invasive, valuable source of vascular information about a woman's future CVD risk after pre-eclampsia. It is hypothesised that pre-eclampsia reflects a complex interplay between impaired placentation and trophoblast dysfunction, and the maternal cardiovascular and other systems.[12 13] In the placenta, defective trophoblast invasion leads to impaired spiral artery remodelling. This could lead to attenuated uteroplacental blood flow causing placental hypoxic-ischaemic and reperfusion damage and poor villous development.[14 15] Histopathological analysis may reveal placental acute atherosis lesions. These occur much more frequently after pre-eclamptic compared with uncomplicated pregnancies; however, the exact prevalence appears to vary depending on sampling and population type.[15–17]

Acute atherosis is identified by the presence of lipid-laden macrophages (foam cells) and fibrinoid necrosis.[18] In the cardiovascular system, foam cells are also a prominent feature of systemic atherosclerotic lesions. These lesions appear to closely resemble each other, which suggests that the presence of placental lesions could reflect a parallel process of developing systemic atherosclerosis.[13 15] Given this similarity, this study hypothesises that women with evident placental vascular changes are at further increased risk of CVD in the future, as evidenced by the presence of coronary subclinical atherosclerosis. In addition, pre-eclampsia is hypothesised to further increase this risk.

Aims and objectives

The primary study aim will be to test the hypothesis that the presence of placental spiral artery acute atherosis relates to coronary subclinical atherosclerosis at two time intervals: at 6-36 months postpartum (short follow-up), and at 10–20 years postpartum (long follow-up). The secondary aim is to determine the influence of pre-eclampsia as a confounder or effect modifier on this relationship for the 6–36 months postpartum group.

Clinical implications

It is widely accepted that women after pregnancies with pre-eclampsia have a higher risk of CVD,[7 8] however, not all of these women will ultimately develop CVD. Specific recommendations allowing identification of these at-risk women are missing. This study has the potential to inform and guide risk stratification after pre-eclamptic pregnancies. If there is a relationship between placental and systemic vascular lesions, placental histopathological analysis could help identify women at the highest risk of future CVD. Early identification of the at-risk population would allow targeted early implementation of targeted CVD prevention strategies, which may include routine CT. The placenta results may further incentivise these women to lead healthier lifestyles and adhere to prevention strategies to minimise the risk of future CVD morbidity and mortality.

METHODS AND ANALYSIS

Study design summary

A feasibility study started in June 2016 and was followed by an unpublished pilot study starting in 2019. Available data from the pilot study will be incorporated into the current study. A longitudinal prospective cohort study will be carried out in the current format between August 2024 and October 2028. Participant recruitment will continue until October 2026. This study has two arms: (1) short follow-up—women with a current pre-eclamptic pregnancy (pre-eclamptic group) or an uncomplicated normotensive pregnancy (uncomplicated group); and (2) long follow-up—women from a historical cohort with a pre-eclamptic pregnancy 10-20 years ago.

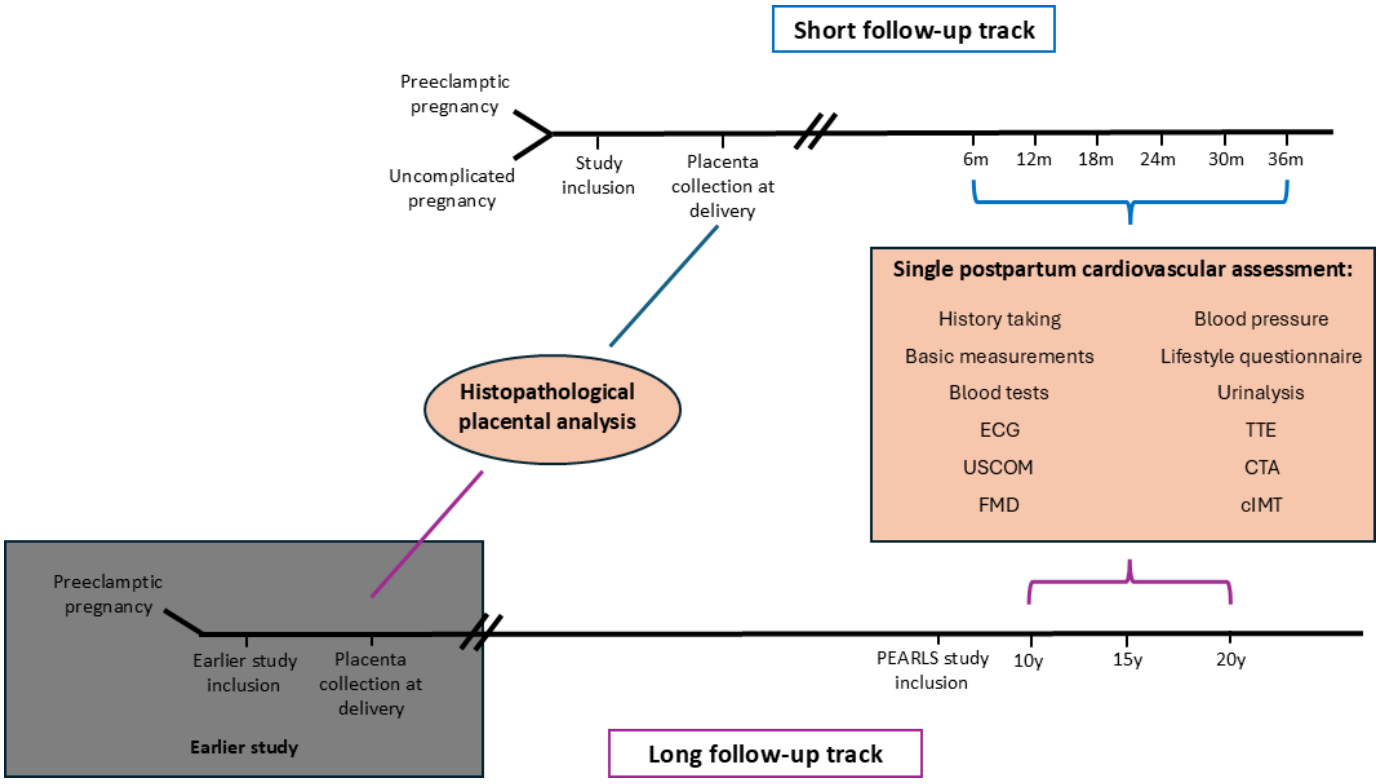

Figure 1 Figure shows the timeline of study participation for both tracks and indicates when study inclusion and investigations are carried out. The lines indicate the histopathological analysis of the placenta. The upper bracket indicates the timeframe for the single postpartum CVD assessment for the short follow-up track whereas the lower bracket indicates the same for the long follow-up track. The left box indicates the contents of the single postpartum cardiovascular assessment. cIMT, carotid intima media thickness; CTA, CT angiography; FMD, flow-mediated dilatation; TTE, transthoracic echocardiography; USCOM, non-invasive ultrasonic cardiac output monitoring.

Both arms will have identical investigations, detailed in online supplemental appendix 1, consisting of placental histopathology and a single postpartum CVD assessment. The CVD assessment includes medical history taking, blood tests, electrocardiography and echocardiography. Additionally, coronary CT angiography (CCTA) focusing on the presence of atherosclerotic plaques and calcium score will be carried out. Short follow-up women will have prospective collection of their placenta, and the single CVD assessment will be carried out 6–36 months postpartum. For the long follow-up, placental tissue is available for reanalysis from their pregnancy 10–20 years ago. The single CVD assessment will take place immediately and corresponds with 10–20 years postpartum. Figure 1 shows an overview of the participation timeline for the short and long follow-up.

Recruitment

For the short follow-up, participants will be recruited during pregnancy in Maastricht University Medical Centre+ from the outpatient clinic or from the inpatient wards. Alternatively, participants can be recruited from external hospitals or midwifery practices if they express an interest. There is close collaboration with Erasmus MC (Rotterdam), University Medical Centre Groningen (Groningen), Amsterdam Medical Centre (Amsterdam) and Radboud University Medical Centre (Nijmegen).

Participant recruitment differs for the long follow-up. Women from a previously studied pre-eclamptic cohort in Radboud University Medical Centre, Nijmegen, The Netherlands will be invited to participate in this study.[19–23] The earlier study included collection of various baseline characteristics and histopathological analyses of the placenta including data on acute atherosis. For participants who choose to take part, the baseline characteristics and placenta data will be made available for use in the current study.

All participants will give written informed consent prior to participating in this study.

Definitions

To account for variation in pre-eclampsia definitions used in recent years and in different hospitals nationally, the traditional definition will be applied in this study. Pre-eclampsia will be defined as hypertension (systolic blood pressure (BP) ≥140 mm Hg and/or diastolic BP ≥90 mm Hg) developed after 20 weeks of pregnancy with de novo proteinuria (≥300 mg/24 hours or PCR >50 mg/mmol) at any gestational age.[24] Uncomplicated pregnancies are pregnancies not complicated by hypertension, growth restriction, placental abruption or preterm birth.

Inclusion and exclusion criteria

For the long follow-up arm, women are included from a historical cohort with a pre-eclamptic pregnancy 10–20 years ago. The historic inclusion criteria for participating in the original cohort study were being admitted with a pre-eclamptic pregnancy to Radboud University Medical Centre, Nijmegen between 2004 and 2010 and and having histopathological analysis of the corresponding placenta. The historic exclusion criteria were multiple pregnancies, incomplete records or incomplete placental samples.[19]

For the current study, the following inclusion and exclusion criteria will be applied to participants in both the short and long follow-up arms. In the pre-eclamptic group, women are included with an index pregnancy complicated by pre-eclampsia, whereas in the uncomplicated group, women are included who have an uncomplicated normotensive index pregnancy. The index pregnancy is the pregnancy for which the placenta has been collected and analysed.

There are several exclusion criteria. In both the pre-eclamptic and uncomplicated groups, women will be excluded if they are under 18 or do not want their test results to be shared with their general practitioner, their specialist(s) or themselves. They will also be excluded if they have an allergy or contraindication to iodinated contrast media, beta-blockers or glyceryl trinitrate (GTN) spray. There are no further exclusion criteria for the pre-eclamptic group. In the uncomplicated group, women cannot have an index pregnancy complicated by hypertension, foetal growth restriction, placental abruption or preterm birth. Before their index pregnancy, women with a history of an uncomplicated pregnancy are excluded if diagnosed with chronic hypertension treated with antihypertensive medication, an autoimmune disorder including systemic lupus erythematosus, Crohn's disease and rheumatoid arthritis or type 1 diabetes mellitus.

For both the pre-eclamptic and uncomplicated groups, women should not be pregnant at the time of the cardiovascular evaluation due to the iodinated contrast media injected for the CCTA. If they are pregnant, they can choose to postpone the postpartum CVD assessment. Breastfeeding is allowed and participants will be instructed to discard breastmilk for 24 hours after completing the investigations. Alternatively, women can also choose to postpone the postpartum assessment.

Description of methods

For the short follow-up, the placenta will be collected directly postpartum. It is either initially stored in a fridge before being transported to the pathology department the next day or transported to the pathology department directly for storage in 4% buffered formaldehyde. The standardised placenta sampling and reporting protocol of the Amsterdam Placental Workshop Group Consensus Statement will be used to guide sampling and analysis of the placentas.[18] In addition to standardising placenta sampling and reporting, it also defines terminology for placental lesions and for placental patterns of disease. To account for sampling error of specific lesions in the placenta, a minimum of eleven biopsies will be taken. Two full-thickness sections will be taken from the umbilical cord: one from the maternal side and one from the foetal side. Two membrane rolls will be taken: one from the rupture edge to the placental margin and the other towards the insertion of the placental disk. Sampling of the thickest part of the membranes is preferred to increase the chance of sampling vasculature. Seven sections will be taken from the parenchyma: one full-thickness section at the umbilical cord insertion site, four central sections of the inner two-thirds of macroscopically normal parenchyma and two peripheral sections from both ends of the longest parenchymal slice. If there are any macroscopic lesions, additional sections will be taken.

The placenta will be analysed by an experienced pathologist. There will be particular focus in the analysis on the presence of acute atherosis. Acute atherosis will be defined by the presence of fibrinoid necrosis, lymphocytic infiltration and presence of lipid-laden macrophages (foam cells). Acute atherosis is a subtype of decidual vasculopathy (DV). DV has six subtypes, which can occur in isolation or in combination. Figure 2 shows the different DV subtypes including acute atherosis: A and B. Acute atherosis, C. Fibrinoid necrosis, D. Lymphocytic infiltration (chronic perivasculitis), E. Absence of spiral artery remodelling, F. Arterial thrombosis and G. Mural hypertrophy.[18]

For the long follow-up group, the placentas were collected and analysed in the earlier study.[19–23] Microscopic reanalysis of available stored placental blocks and slides will be carried out for the current study by an experienced pathologist following the Amsterdam Placental Workshop Group Consensus Statement.[18]

The single postpartum CVD assessment will be identical for both the short and long follow-up. Various investigations will be carried out to gain insight into different CVD parameters (Figure 1). All participants will be in a fasted state when they undergo the CVD assessment.

Participants will be asked about their medical history, drug history including intoxications, obstetric history and family history. They will complete a Short QUestionnaire to ASsess Health-enhancing physcial activity (SQUASH) lifestyle questionnaire.[25 26] Basic measurements will be taken including weight, height, waist and hip circumference and BP. The median brachial artery BP will be recorded using a 30 minute measurement with 3 minute intervals using a semiautomatic oscillometric device (Dinamap Vital Signs Monitor 1846, Critikon, Tampa, Florida).[27]

Fasting blood and urine samples will be taken. Blood tests will include haemoglobin, mean corpuscular volume, renal function, liver function, thyroid function, glucose, insulin, HbA1c, cholesterol and NT-pro-BNP. Additional blood samples will be stored at −80°C. These samples will be used in future to determine biomarkers and perform DNA analyses based on new insights, which are anticipated in the coming years.

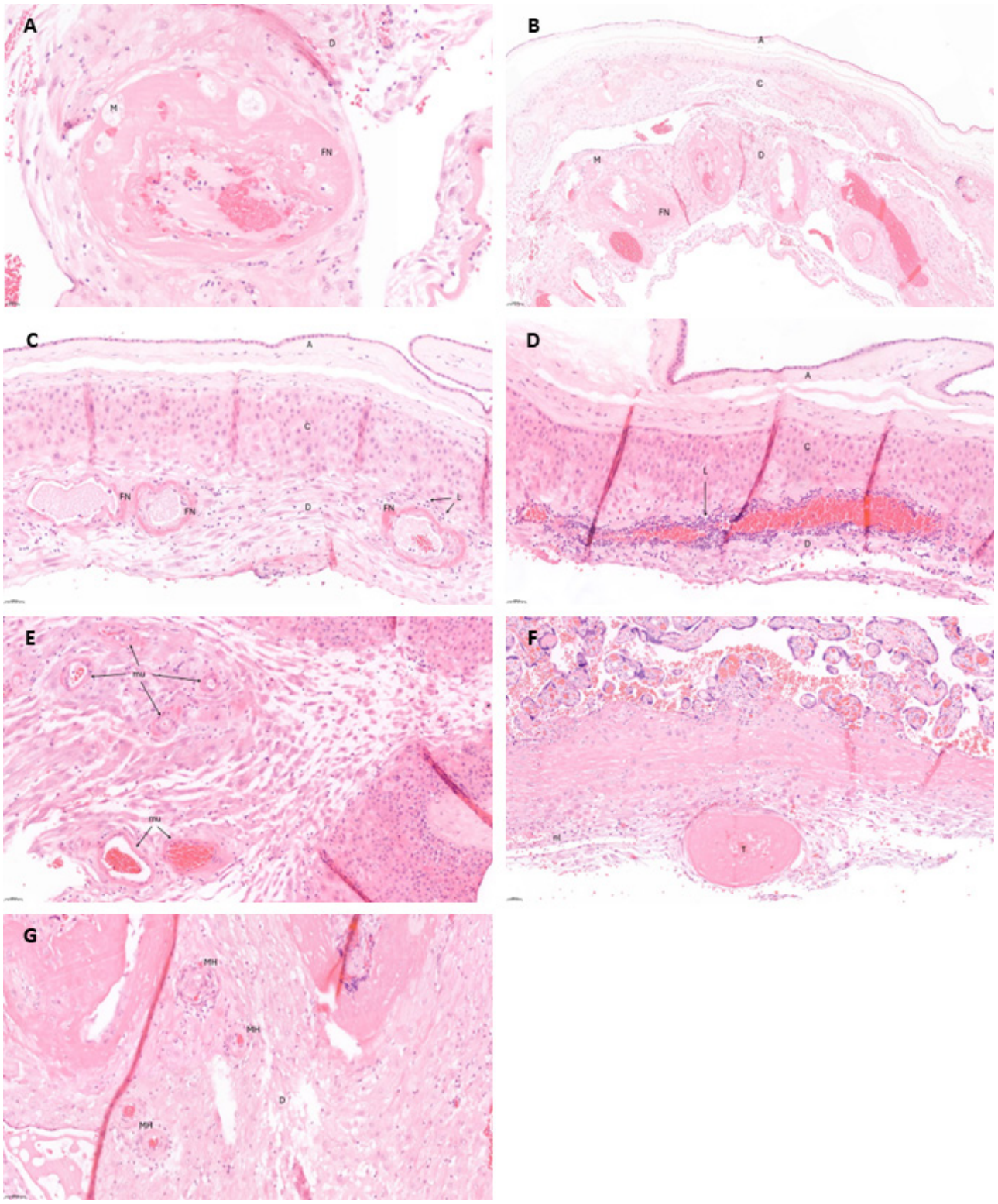

Figure 2 Figure showing different subtypes of decidual vasculopathy in the maternal blood vessel of the placenta. (A) *Acute atherosis* consisting of fibrinoid necrosis (FN) and foamy macrophages (M) in the decidua parietalis (D). (B) *Acute atherosis* consisting of FN and foamy macrophages (M) shown in the placental membranes composed of amnion (A), chorion (C) and decidua parietalis (D). (C) FN with maternal blood vessels surrounded by lymphocytes (L) in the placental membranes composed of amnion (A), chorion (C) and decidua parietalis (D). (D) *Chronic perivasculitis/lymphocytic infiltration* around one maternal blood vessel which is cuff-like surrounded by lymphocytes (L) in the placental membranes composed of amnion (A), chorion (C) and decidua parietalis (D). (E) *Incomplete remodelling* of the maternal blood vessels in the decidua basalis characterised by a persistent muscular layer (mu) of the vessel wall. (F) *Occluding thrombus* (T) in a maternal blood vessel surrounded by normally remodelled blood vessels (nl) in the decidua basalis. (G) *Mural hypertrophy* (MH) characterised by a wall thickness >30% of diameter of the maternal blood vessels in the decidua basalis (D).

Cardiac function, structure and geometry will be investigated using ECG, transthoracic echocardiography (TTE) and non-invasive ultrasonic cardiac output monitoring (USCOM). TTE and USCOM measurements will be carried out by an experienced echocardiographer blinded to the patients' history.

The procedure for TTE follows the most up to date guidelines whereby images will be obtained in a left lateral position.[28] Images will be recorded to allow offline analysis. There will be a quantitative assessment of cardiac dimensions and left ventricular systolic function. Left ventricular systolic function will also be assessed according to current guidelines.[29] Right ventricular function will be assessed in different ways including using the tricuspid annular plane systolic excursion and tissue Doppler imaging. Diastolic function will also be explored according to current guidelines, including using speckle tracking for strain analyses, and E/A and E/e' ratios.[29]

For the USCOM measurement, the participant will be in the supine position. The probe will be placed in the suprasternal notch and directed across the aortic valve to acquire a spectral Doppler flow profile displayed as a time velocity plot. The optimal flow profile will be recognised by a triangular shape where the base covers the full systolic width, and the sides are straight and continuous before converging into a sharp peak. Once the optimal flow profile and highest velocity (minimum of 1 m/s) are obtained, the trace will be frozen. Automatic tracing of the flow profiles will allow calculation of the stroke volume (product of the velocity–time integral and the cross-sectional area of the aortic valve). The value for the cross-sectional area of the aortic valve will be automatically estimated using the height. Three serial measurements will be carried out to calculate cardiac output. The velocity–time integral is calculated for each individual measurement and will usually be calculated by recording all cardiac cycles during four respiratory cycles. The number of cardiac cycles recorded depends on the heart rate. USCOM will record more cardiac cycles than TTE, enabling more comprehensive analyses of the cardiac cycle accounting for both the baroreceptor-mediated fluctuation and the full respiratory cycle.[30] Cardiac output will be calculated from the product of the heart rate and the stroke volume.

A CT scan of the heart will be performed using a third-generation dual-source CT scanner (Somatom Force, Siemens Healthineers, Forchheim Germany) or a photon counting CT scanner (NAEOTOM Alpha, Siemens, Healthineers, Forchheim Germany). ECG and heart rate will be monitored during CCTA. Prior to CCTA, a standardised non-contrast scan of the heart will be performed (eg, Agatston score) to determine the overall burden of coronary calcification. For this score, calcified plaques in each coronary artery are identified by voxels ≥1 mm^2 with a minimum threshold of 130 Hounsfield units.[31] Figure 3 shows examples of CCTA images and the presence of calcified plaque in the left anterior descending artery. The non-contrast scan of the heart is followed by a CCTA after administration of iodinated contrast media. In patients with a stable heart rate <65 bpm, a prospectively ECG-triggered 'high pitch' spiral protocol will be used. In patients with a stable heart rate between 60 bpm and 90 bpm, a prospectively triggered 'adaptive sequence' protocol will be used (prospective sequential data acquisition). Retrospective gating will be used in patients with an irregular heart rate or a heart rate >90 bpm. Oral or intravenous metoprolol will be given shortly before the scan, if deemed necessary. GTN will be given to all patients for vasodilation prior to the scan, in the absence of contraindications. Images will be reviewed in consensus by a dedicated cardiovascular radiologist and cardiologist according to the Society of Cardiovascular Computed Tomography guidelines.[31] The presence and extent of CAD, and its subsequent plaque morphology and degree of stenosis will be described.

The carotid arteries will be assessed bilaterally using B-mode ultrasound following an identical procedure for both carotid arteries. Initially, the systolic and diastolic flow is measured at the proximal common carotid, middle common carotid, distal common carotid, proximal internal carotid and the proximal external carotid. Afterwards, a straight section of the proximal common carotid, middle common carotid, distal common carotid and carotid bulb will be visualised. For each section, the distance between the border of the luminal intima and the border of the media adventitia will be measured to determine the carotid intima media thickness. Subsequently, the presence of plaque at the carotid bulb will be determined; if present, the length, thickness, circumference and area of the plaque will be measured.

In addition, the intima media thickness will also be measured in a different manner. A straight segment of the common carotid artery will be selected just before the carotid bulb or bifurcation and a recording is made of five to six cardiac cycles. This recording will be analysed postcapture using a vidArt program developed by professor A. Hoeks (Matlab R2013b, The Math-works Massachusetts). This program can identify the walls and borders of the artery by tracking these automatically. This can be adjusted manually if recording quality is poor or artefacts are present. Analysis using this program allows intima media thickness to be measured and also allows additional variables to be generated such as the SD of the intima media thickness and the homogeneity of the measurements.

Flow-mediated dilatation (FMD) measured will evaluate endothelial function using a brachial artery ultrasound. FMD is the endothelium-mediated dilatation response to a sudden increase in shear stress. The brachial artery diameter will be measured continuously over 9 min. A sphygmomanometer cuff will be placed on the dominant forearm of the subject. During the first 3 min baseline diameter and baseline blood flow, velocity of the brachial artery will be determined. Subsequently, the cuff on the forearm will be inflated up to 200 mm Hg and will remain at this pressure for 5 min to cause distal (forearm)

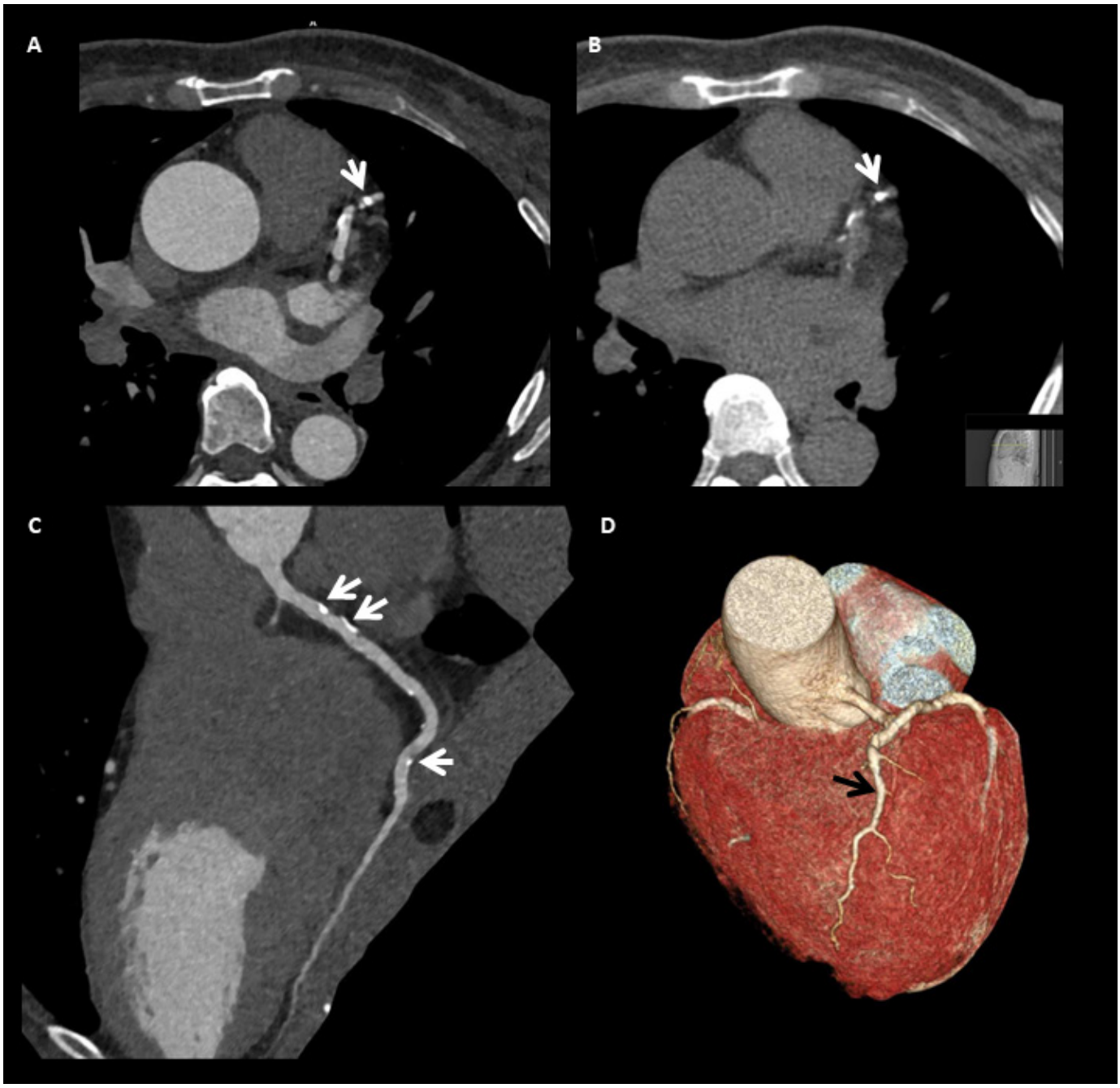

Figure 3 (A) Transverse CTA image of the heart depicting the calcified plaque in the proximal LAD (white arrow). (B) Non-contrast scan of the heart to determine the presence and extent of calcified plaques. White arrow indicates a calcified plaque in the LAD. (C) Curved MPR image of the LAD. The white arrows indicate the presence of calcified plaques. (D) 3D volume rendering image of the heart. Black arrow indicates the LAD. CTA, CT angiography; LAD, left anterior descending artery; MPR, multiplanar reformation.

hypoxia. The cuff will then be rapidly deflated and as a response to the hypoxia, vasodilatation of the vessels will occur. The arterial diameter and blood flow velocity will be measured for another 5 min. During the measurement, dual mode ultrasound images will be recorded. Afterwards, a new 3 min rest recording will be carried out followed by one spray of sublingual GTN which will be administered. GTN induces vasodilatation by converting into nitric oxide in the vascular wall. Recording of the vessel for 10 min after GTN administration enables discrimination of endothelial-dependent dilation and endothelial-independent dilation.

Power calculation

Primary aim

The sample size is powered based on the primary aim, which looks at the presence or absence of placental acute atherosis and subsequent differences in the prevalence of coronary artery subclinical atherosclerosis at both time intervals. No studies have directly related acute atherosis

to subclinical atherosclerosis prevalences, however, we expect acute atherosis to relate to higher prevalences of subclinical atherosclerosis.[15 19 32 33] Given that there is a higher rate of acute atherosis after pre-eclamptic pregnancies and a very low rate after uncomplicated pregnancies,[15–17] the prevalence of atherosclerosis after complicated and uncomplicated pregnancies will be used as a proxy on which to base-estimated prevalences for the presence and absence of placental acute atherosis, respectively.

For the short follow-up arm of this study with a follow-up of 6-36 months postpartum, the average age range will be assumed to be between 25 and 40 years. In this age range, for atherosclerosis, prevalence after pre-eclamptic pregnancies is roughly 17% and after uncomplicated pregnancies, the prevalence is 12%.[34–36] To account for the proportion of acute atherosis in each respective group, the prevalence of subclinical atherosclerosis in the acute atherosis group is expected to be higher at 30%, whereas the prevalence is expected to be lower at 10% in the non-acute atherosis group. For a two-sided power calculation with a power of 80% and an alpha of 0.05 with a 1:1 ratio, this study will need a total of 138 women in the short follow-up arm: 69 women with acute atherosis and 69 women without acute atherosis.[37 38]

For the long follow-up arm of this study with a follow-up of 10–20 years postpartum, the sample size is powered in a similar manner as the short follow-up arm with the same assumptions. The average age range will be assumed to be between 40 and 60 years. The subsequent prevalence of atherosclerosis after complicated pregnancies is roughly 35% and after uncomplicated pregnancies is 25%.[39–41] To account for the proportion of acute atherosis in each respective group, the prevalence of subclinical atherosclerosis in the acute atherosis group is expected to be much higher at 50%, whereas the prevalence is expected to be lower at 20% in the non-acute atherosis group. For a two-sided power calculation with a power of 80% and an alpha of 0.05 with a 1:1 ratio, this study will need a total of 88 women in the long follow-up arm: 44 women with acute atherosis and 44 women without acute atherosis.[37 38]

Secondary aim

For the secondary aim which determines the influence of pre-eclampsia as a confounder for the short follow-up arm, the rule of thumb of 10 events per determinant will be used to determine the power required to explore this.[42] This aim would require the total inclusion of 83 women, based on the rule of thumb and an estimated overall prevalence of atherosclerosis of 12% for this age range.[34–36] As this is lower than the power for the primary aim, the primary aim will determine the required study sample size for the short follow-up arm.

Statistics

The latest version of SPSS software will be used for the analyses. Missing values will be supplemented using source data where possible and imputation will be considered when appropriate. The distribution of continuous data will be evaluated for normality visually using histograms, P-P and Q-Q plots. Data will be expressed as mean and SD for normally distributed data and as median and IQR for skewed data. Categorical data will be presented as a number with percentage. Between-group differences will be statistically evaluated as appropriate. Univariable linear and logistic regression analysis will be used to investigate the prevalence of acute atherosis and subclinical atherosclerosis in formerly pregnant women. Potential confounding or effect-modifying effect of pre-eclampsia history on the association between acute atherosis and atherosclerosis will be evaluated by multivariable regression analyses as well as the effect of other confounders and effect modifiers including body mass index, age and family history of CVD. To adjust for confounders, multivariable analyses will be performed for both outcomes. All p values are two tailed and a p value <0.05 will be considered statistically significant.

Patient and public involvement

The patient organisation ‘HELLP stichting’ has been involved with the study design of this study.

ETHICS AND DISSEMINATION

This protocol has been approved by the Medical Ethics Committee at Maastricht University Medical Centre+ (NL52556.068.15/METC152019). The study protocol was designed in accordance with the principles of the Good Clinical Practice guidelines of the European Community and with the Declaration of Helsinki.

Results will be shared by publishing in peer-reviewed journals and by presentations at various conferences.

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