The Placenta as a Predictor for Future Cardiovascular Health following Placenta-Mediated Diseases

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Preface

Abstract

Introduction: The placenta is essential for fetal development and pregnancy prolongation. Its dysfunction can lead to short and long-term health consequences for mother and child. A subset of diseases resulting from placental dysfunction have been collectively termed placental-mediated diseases (PMD) - which includes the common and serious hypertensive disorder of pregnancy preeclampsia (PE), among others. PMDs are independent risk factors for maternal cardiovascular disease (CVD) in later life. This thesis presents a multi-pronged body of work, which includes: 1) A systematic review, aimed at summarizing the current state of knowledge on the risk for future maternal CVD after PMDs; 2) A cohort study, aimed at assessing the utility of placenta pathology examination at delivery to identify women at high lifetime risk for CVD following PE; and 3) An assessment of an immunohistochemistry screening panel for 3 placenta protein markers of interest, aimed at determining if these markers can accurately identify women deemed to be at high-risk for future CVD following a PE pregnancy.

Methods: 1) We searched 4 databases for observational studies evaluating clinical and biochemical markers of CVD risk and/or a subsequent calculated risk score based on these parameters in women with a history of PMD. We excluded interventional studies and studies measuring these outcomes during or prior to pregnancy. 2) A cohort study was established across two clinical sites (Kingston, Ottawa), in which patients with PE (N=85) underwent cardiovascular risk assessments at 6-months postpartum. The placentas from these pregnancies also underwent detailed placenta histopathology examination to determine the presence, absence, and severity of 35 distinct placental lesions. The associations between distinct placental lesions and estimated lifetime cardiovascular risk were evaluated by odds ratios (OR) and receiver operator curve

analysis (ROC). 3) Immunohistochemistry (IHC) analysis was performed on placental samples from a subset of the previously described cohort (N=41; Ottawa site only). Protein expression for FLT-1, ENG, and CD68 was quantified. Using a multivariate logistic regression model, the association between placenta protein expression, with and without clinical and placenta pathology findings, and cardiovascular risk was assessed.

Results: 1) The search yielded 11,039 articles of which 104 met our inclusion criteria. All PMD types demonstrated evidence of increased CVD risk markers at varying timepoints postpartum. At least one study per PMD type had non-optimal measures of systolic blood pressure, BMI, and total cholesterol. 2) In the analysis of placenta pathology lesions within the cohort of individuals with PE, lesions of maternal vascular malperfusion (MVM) were found to be associated with elevated life-time risk for maternal CVD at 6 months postpartum (OR: 3.10[1.20-7.92]). We also found that adding these lesions to a logistic regression model improved the predictive accuracy for elevated maternal lifetime CVD risk (AUC: 73.0, sensitivity: 78.4%, specificity: 51.6%). 3) Individually, no significant differences were found in FLT-1, ENG, and CD68 expression between the individuals deemed to be at high and low-risk for lifetime CVD. Although, when added to a model that included placenta pathology lesions and clinical data the predictive accuracy for elevated maternal lifetime CVD risk increased (AUC: 1.0, sensitivity: 100%, specificity: 100%).

Conclusions: In conclusion, this thesis provides further evidence of the utility of assessing placenta features in the prediction of future maternal CVD. This is evidenced by the association between PMDs, placental pathology, and placental protein biomarkers with elevated risk profiles for lifetime CVD. Thus, specific placental phenotypes of PMDs may be at increased risk for CVD.

The use of placental data should be further explored as a triage strategy to identify these high-priority of women following delivery.

Résumé

Introduction : Le placenta est essentiel pour le développement fœtal et la prolongation de la grossesse. Cela dit, sa dysfonction peut mener à des conséquences courtes et long-termes sur la santé de la mère et de l'enfant. Un sous-ensemble de maladies résultant de cette dysfonction ont été classifiées comme des pathologies placentaires (PP). Ceci inclue la prééclampsie (PE), un trouble hypertensif de la grossesse, entre autres. Les PP sont des facteurs de risques indépendantes pour les maladies cardiovasculaires (MCV) futures chez la mère. Cette thèse adopte une approche à plusieurs volets, qui inclue : 1) Une revue systématique, qui résume la littérature sur le risque du MCV futures chez la mère après des PP; 2) Une étude de cohorte qui évalue l'utilité des examens de pathologie placentaire après l'accouchement à identifier des femmes ayant un plus haut risque pour la MCV après la PE; et 3) Une évaluation d'un panneau d'immunohistochimie pour trois marqueurs de protéine d'origine placentaire pour l'identification des femmes à haut risque pour la MCV après la PE.

Méthodologie : 1) Nous avons effectué une recherche systématique de 4 bases de données pour des études observationnelles qui évalue les marqueurs cliniques et biochimiques du risque future pour la MCV pour les femmes après des PP. Nous avons aussi ciblé des études qui ont calculé un score de risque pour la MCV avec ces marqueurs. Nous avons exclu des études interventionnelles et des études qui ont évalué ces données pendant ou avant la gestation. 2) Nous avons effectué une étude de cohorte à deux sites cliniques (Kingston, Ottawa), dans laquelle les patientes atteintes de PE (N=85) ont fait l'objet d'une évaluation du risque cardiovasculaire à 6 mois après

l'accouchement. Leurs placentas ont également fait l'objet d'un examen histopathologique détaillé afin de déterminer la présence, l'absence et la gravité de 35 lésions placentaires distinctes. L'association entre ces lésions placentaires et le risque cardiovasculaire estimé au cours de la vie ont été évaluées par des rapports de cotes (RDC) et une analyse de la courbe d'opérateur récepteur (ROC). 3) Une analyse immunohistochimique (IHC) a été réalisée sur des échantillons placentaires provenant d'un sous-ensemble de la cohorte décrite précédemment (N=41 ; site d'Ottawa uniquement). L'expression protéique de FLT-1, ENG et CD68 a été quantifiée. À l'aide d'un modèle de régression logistique multivarié, l'association entre l'expression des protéines placentaires, avec ou sans résultats cliniques et de pathologie placentaire et le risque cardiovasculaire a été évaluée.

Résultats : 1) Notre recherche systémique a produit 11,039 articles dont 104 ont satisfait nos critères d'inclusion. Tous les types de PP ont démontré de l'évidence des élévations de marqueurs de risque pour le MCV dans la période postpartum. Au moins une étude par type de PP a trouvé des mesures non-optimal de la pression sanguine systolique, de l'IMC et du cholestérol totale. 2) Dans l'analyse des lésions de la pathologie du placenta au sein de la cohorte des individus atteints du PE, il a été constaté que les lésions de malperfusion vasculaire d'origine maternelle (MVM) étaient associées à un risque élevé de MCV maternel à 6 mois postpartum (RDC: 3,10 [1,20-7,92]). Nous avons également constaté que l'inclusion de ces lésions à un modèle de régression logistique améliorait la performance prédictive d'un risque élevé de MCV maternelle (AUC: 0,73; sensibilité: 78,4%; spécificité: 51,6%). 3) Individuellement, aucune différence significative n'a été constatée dans l'expression de FLT-1, ENG et CD68 entre les individus considérés comme présentant un risque élevé et faible de MCV à vie. Cependant,

lorsqu'ils ont été ajoutés à un modèle incluant les lésions pathologiques du placenta et les données cliniques, la précision prédictive d'un risque élevé de MCV à vie chez la mère a augmenté (AUC: 1,0; sensibilité: 100 %; spécificité: 100 %).

Conclusions : En conclusion, cette thèse fournit des preuves supplémentaires de l'utilité de l'évaluation des caractéristiques du placenta dans la prédiction des MCV futures chez la mère. Ceci est mis en évidence par l'association entre les PP, l'histopathologie placentaire et les marqueurs de protéines placentaires avec des profils de risque élevés pour le MCV. Ainsi, des phénotypes placentaires spécifiques des PP pourrait présenter un risque plus élevé pour le MCV. L'utilisation des données placentaires devraient donc être étudiée en plus en tant que stratégie de triage pour identifier ces femmes à haut risque après l'accouchement.

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Authorship Contribution

The following outline lists the contributions of various authors to the manuscript presented in this thesis.

Manuscript 1: *A systematic review of the association between placenta-mediated diseases during pregnancy and estimated maternal risk for cardiovascular disease*

Dr. Shannon Bainbridge was the principal investigator for this study. The study was conceptualized by Dr. Shannon Bainbridge and Erika Mery. Nigèle Langlois, Erika Mery, Eden Clark-Campbell, and Dr. Shannon Bainbridge wrote the search strategy. Erika Mery, Eden Clark-Campbell, and Hannah Poisson performed title and abstract screening of 7599 articles retrieved from the literature search of all databases. Erika Mery, Eden Clark-Campbell, and Hannah Poisson proceeded with a full-text review of screened-in articles, data extraction, and assessment of bias. The manuscript was prepared by Erika Mery and Eden Clark-Campbell and revised by Dr. Shannon Bainbridge.

Manuscript 2: *Placental pathology as a tool to identify women for postpartum cardiovascular risk screening following preeclampsia: a preliminary investigation*

Dr. Shannon Bainbridge and Dr. Graeme Smith were the principal investigators for the study. The study was conceptualized by Dr. Shannon Bainbridge, Dr. Graeme Smith, Dr. Samantha Benton, and Dr. Laura Gaudet. Alysha Harvey and Jessica Pudwell were responsible for organizing participant recruitment and data collection at the respective study sites. Dr. David Grynspan, Dr. Samantha Benton and Erika Mery performed placental pathology data collection. Statistical analysis and manuscript preparation was completed by Dr. Samantha Benton and Erika Mery. The manuscript was revised by Dr. Shannon Bainbridge.

Manuscript 3: *Placental protein expression of fms-like tyrosine kinase-1 (FLT-1), endoglin (ENG), and cluster of differentiation 68 (CD68) and future maternal cardiovascular risk profiles following preeclampsia*

Dr. Shannon Bainbridge and Dr. David Grynspan were the principal investigators for the study. The study was conceptualized by Dr. Shannon Bainbridge, Dr. David Grynspan and Dr. Samantha Benton. Alysha Harvey and members of the OMNI team were responsible for organizing participant recruitment and data collection. The Department of Pathology at the Children's Hospital of Eastern Ontario processed the placental tissues and generated all study histology slides. Dr. Goutham Vasam and Erika Mery performed the immunohistochemistry for the three proteins of interest. Quantification, statistical analysis, and manuscript preparation was completed by Shrreya Sudade and Erika Mery. The manuscript was revised by Dr. Shannon Bainbridge.

Ethics Statement

Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans Course on Research Ethics (TCPS 2:CORE) was completed on July 9th 2018. All certificates of ethics approval, renewal and study closure relevant to the current thesis and TCPS 2:CORE completion are included in Appendix A. This thesis project involved recruitment and sample/data collection from a number of clinical sites and as such different aspects of this study were granted research ethics approval by the University of Ottawa Research Ethics Board, the Ottawa Health Science Network (OHSN)-REB, the Children's Hospital of Eastern Ontario (CHEO)-REB and Queen's University Health Science REB. Reference to the specific ethics protocols relevant to the different manuscripts presented in this thesis are highlighted below. Erika Mery was added to these ongoing REB approvals as a student researcher to perform an analysis of the samples and data collected at the various clinical sites.

Manuscript 2: *Placental pathology as a tool to identify women for postpartum cardiovascular risk screening following preeclampsia: a preliminary investigation*

University of Ottawa REB – "DREAM: Determining the Risk Elevation After Maternity"; Protocol # A04-15-01

- Original approval date: April 1, 2014
- Renewal dates: April 10, 2019; March 17, 2018; March 20, 2017; March 18, 2016; Sept 22, 2015
- Study closure date: June 3, 2020

OHSN-REB – "DREAM – Determining the Risk Elevation after Pregnancy"; Protocol # 20140799-01H

- Original approval date: March 19, 2015
- Renewal dates: Feb 27, 2019; March 1, 2018; March 1, 2017; March 10, 2016, July 23, 2015; April 22, 2015
- Study closure date: Feb 15, 2020

CHEO-REB – "DREAM: Determining the Risk Elevation After Maternity"; Protocol # 14/210X

- Original approval date: March 23, 2015
- Renewal dates: Jan 17, 2019; Jan 31, 2018; March 15, 2017; Jan 21, 2016
- Study closure date: February 13, 2020

Queen's University Health Science REB – "Using placental lesions to identify women at highest risk of future cardiovascular disease following preeclampsia"; Protocol # OBGY-295-16

- Original approval date: Jan 13, 2017
- Renewal dates: April 16, 2021, Dec 3, 2020; Dec 17, 2019; Jan 8, 2019; July 11, 2018

Manuscript 3: *Placental protein expression of fms-like tyrosine kinase-1 (FLT-1), endoglin (ENG), and cluster of differentiation 68 (CD68) and future maternal cardiovascular risk profiles following preeclampsia*

University of Ottawa Research Ethics Board – "A Novel Histopathology Diagnostic Antibody Panel Capable of Identifying Subclasses of Placental Disease in Preeclampsia"; Protocol # A04-16-05

- Original approval date: April 25, 2016
- Renewal dates: Jan 28, 2019; Feb 15, 2018; March 17, 2017, Jan 28, 2020

OHSN-REB – “A novel histopathology diagnostic antibody panel capable of identifying distinct subclasses of placental disease in preeclampsia”; Protocol # 20150941-01H

- Original approval date: May 2, 2016
- Renewal dates: April 10, 2019; April 25,2018; April 21, 2017; June 20, 2016
- Study closure date: April 27, 2020

CHEO-REB – “A novel histopathology diagnostic antibody panel capable of identifying distinct subclasses of placental disease in preeclampsia: Protocol # 15/166X

- Original approval date: Jan 27, 2016
- Renewal date: Dec 9, 2019; Nov 19, 2018; Nov 20, 2017; Jan 29, 2016
- Study Closure: December 17, 2021

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List of Abbreviations

AUC	Area Under the Curve
BMI	Body Mass Index
BP	Blood Pressure
CC	Cytotrophoblast Cells
CD68	Cluster of Differentiation 68
CI	Confidence Interval
CHEO	Children's Hospital of Eastern Ontario
CVD	Cardiovascular Disease
CVR	Cardiovascular Risk
DAB	3,3'-Diaminobenzidine
ENG	Endoglin
EVT	Extravillous Cytotrophoblast
HDL	High Density Lipoprotein
HDP	Hypertensive Disorder of Pregnancy
H&E	Hematoxylin and Eosin
hsCRP	High Sensitivity C-Reactive Protein
INHBA	Inhibin Subunit Beta A
IL-6	Interleukin 6
FGR	Fetal Growth Restriction
IV	Intervillous Space
GDM	Gestational Diabetes Mellitus

LEP	Leptin
LDL	Low Density Lipoprotein
MVM	Maternal Vascular Malperfusion
OR	Odds Ratio
PE	Preeclampsia
PIGF	Placental Growth Factor
PTB	Preterm Birth
ROC	Receiver Operator Curve
SC	Syncytiotrophoblasts Cells
sFLT-1	Soluble FMS-Like Tyrosine Kinase-1
FLT-1	FMS-Like Tyrosine Kinase-1
SGA	Small for Gestational Age
TNF-a	Tumor Necrosis Factor Alpha
TCPS 2: CORE	Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans Course on Research Ethics

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Thesis Outline

Chapter 1: Introduction

The introduction provides a review of the relevant literature, study rationale, overarching hypothesis, and research objectives for this thesis.

Chapter 2 (Manuscript 1): *A systematic review of the association between placenta-mediated diseases during pregnancy and estimated maternal risk for cardiovascular disease*

This first manuscript addresses research aim 1, sub-aim 1, 2, and 3. This article presents a systematic review of the literature related to the estimated risk of cardiovascular disease after diagnosis of placental-mediated diseases during pregnancy. This manuscript is prepared for submission to the Journal of Clinical Medicine special issue on New Insights into Pregnancy Complications.

Chapter 3 (Manuscript 2): *Placental pathology as a tool to identify women for postpartum cardiovascular risk screening following preeclampsia: a preliminary investigation*

This second manuscript addresses research aim 2, sub-aim 1 and 2. This article demonstrates how placental lesions can identify women with an elevated lifetime risk for cardiovascular disease following preeclampsia. This manuscript has been published (*J. Clin. Med.* 2022 Mar;11(6):1576)

Chapter 4 (Manuscript 3): *Placental protein expression of fms-like tyrosine kinase-1 (FLT-1), endoglin (ENG), and cluster of differentiation 68 (CD68) and future maternal cardiovascular risk profiles following preeclampsia*

This third manuscript addresses research aim 3, sub aim-1, 2, and 3. This article demonstrates the association between subclasses-specific protein markers assessed via immunohistochemistry

analysis of FLT-1, ENG, and CD68 expression and elevated risk for lifetime cardiovascular disease in women with a history of preeclampsia. This manuscript is prepared for submission to Placenta.

Chapter 5: General Discussion and Conclusions

The discussion critically interprets the findings of the thesis project results and highlights the broader impact of this body of work in this field of study. This section also discusses the relevant study limitations, future directions, interdisciplinarity of the project and concluding remarks.

Chapter 1

Introduction

1.1 The Human Placenta

Placental Anatomy and Physiology

The placenta is a highly specialized organ that sustains fetal growth and development throughout pregnancy [1]. Its main functions are metabolic, immunological, and endocrine [1]. Acting as a bridge between maternal and fetal circulation, it mediates the transfer of essential nutrients, metabolites, gases, and waste products [1]. It also protects the fetus from certain xenobiotics, bacteria, and maternal diseases by preventing direct contact between the two circulations via the placental barrier [1]. Yet, some maternal antibodies can pass, providing the fetus with passive immunity for potential infections [1]. Furthermore, it serves an endocrine function by synthesizing hormones essential to the prolongation of pregnancy and fetal development [1].

The placenta is composed of tissues derived from both maternal and fetal origin [1]. As such, it can be divided into two distinct plates: chorionic (fetal surface) and basal (maternal surface) [2]. The chorionic plate is covered with amnion, an avascular membrane which plays a key role in amniotic fluid regulation [2]. It is also the site of umbilical cord insertion, connecting the placenta to fetal circulation [2]. Typically, the umbilical cord is composed of two umbilical arteries and one umbilical vein [2]. These umbilical vessels radiate into chorionic vessels within the chorionic plate and eventually into fetal capillaries within placental villi [2]. In contrast, the basal plate is primarily composed of extravillous trophoblast, fibrinoid, and decidua [2]. It also contains the uterine spiral arteries and utero-placental veins, connecting the placenta to maternal circulation [2]. The intervillous space is found between these plates and contains the placental villi [1]. These tree-like structures are the functional units of the placenta [1]. They contain fetal

blood vessels surrounded by a protective placental barrier [1]. By extensively branching, contact area for maternal-fetal exchange is optimized within the terminal villi regions [1]. To allow for a bidirectional exchange, the placenta contains two circulations [3]. The fetal-placental circulation carries fetal blood into the fetal capillaries within terminal villi by the umbilical arteries and back towards the fetus by the umbilical vein [3]. In contrast, the maternal utero-placental circulation carries maternal blood into the intervillous space by the uterine spiral arteries and back towards maternal circulation by the endometrial veins [3].

The placenta can be further divided into two functional compartments: extravillous and villous [1]. The extravillous compartment is the site of uterine attachment whereas the villous compartment is the site of maternal-fetal exchange [1]. Villous stem cell cytotrophoblasts can differentiate into functional cells within either compartment [1]. Differentiation via the extravillous trophoblast (EVT) pathway leads to the formation of endovascular trophoblasts and interstitial trophoblasts which mediate the maternal uterine adaptations to pregnancy [1]. Endovascular trophoblasts are involved in uterine spiral artery remodelling whereby blood vessel resistance is reduced to allow for the increase in blood flow necessary to sustain fetal development [1]. Interstitial trophoblasts invade into the decidua of the uterine wall and play an important role in the establishment and maintenance of maternal immune tolerance to the developing feto-placental unit [4]. Differentiation via the villous pathway leads to syncytiotrophoblasts (SC). SC form a single multinucleated outer layer that envelops the placental villi, forming the placental barrier between maternal blood in the intervillous space and fetal blood within the villi vasculature [1]. SC are the site of maternal-fetal exchange as well as

immunological and endocrine functions of the placenta [1]. A schematic representation of the placental surfaces and compartments is included in **Figure 1.1.**

Placenta-Mediated Diseases

Hence the importance of its role, a healthy placenta is key to a healthy pregnancy [1]. As such, placental dysfunction is one of the main causes of obstetrical complications and adverse perinatal and maternal health outcomes [5,6]. Its effects can also extend beyond the peripartum period, with associated life-long health risks for both mother and child [7]. In addition, placental dysfunction in an index pregnancy is associated with recurrence and complications in future pregnancies [8]. To describe diseases resulting from abnormal placental development or placental dysfunction, the term placenta-mediated diseases has emerged within the literature [6-10]. The umbrella term includes many of the most common and serious obstetrical complications seen today, including: preeclampsia (PE), Hemolysis Elevated Liver Enzymes and Low Platelets (HELLP) syndrome, fetal growth restriction, gestational diabetes mellitus, placental abruption, stillbirth, and spontaneous pre-term birth [6-10]. Collectively, these diseases can affect up to one third of all pregnancies. Globally, their incidence is increasing in recent years [6]. While more research on the cause(s) of placenta-mediated diseases is warranted, some possible shared mechanisms include deficient placentation, incomplete spiral artery remodelling, oxidative stress, placental mitochondrial dysfunction, abnormal development of chorionic villi, and placental inflammation [10,11].

Placental Histopathology

In the event of an adverse pregnancy outcome resulting from a placenta-mediated disease, a clinical placental pathology examination following delivery is recommended by

pathology associations worldwide [12]. This examination comprises of a detailed assessment of placental gross anatomy and histological examination of the umbilical cord, membranes, and villous parenchyma [9]. During the gross assessment, the trimmed placenta weight is recorded along with measurements and observations of the placental disk, membranes, and umbilical cord [9]. For singleton pregnancies, 3 full thickness biopsies of the villous parenchyma are recommended for further microscopic assessment [9]. These sections are mounted and stained with hematoxylin and eosin (H&E) [9]. Upon microscopic observation, perinatal pathologists can assess visual patterns and diagnose a variety of histopathological lesions [9]. Findings from the gross and microscopic assessments are typically summarized in a narrative pathology report [9].

In 2015, the Amsterdam Placental Workshop was held to improve global standardization of placental pathology reporting by generating a consensus statement from leading experts [13]. This resulted in the organization of individual placental lesions into the following 10 categories: Maternal Vascular Malperfusion (MVM), Maternal Decidual Arteriopathy, Implantation Site Abnormalities, Evidence of Intrauterine Infection, Evidence of Placenta Villous Maldevelopment, Fetal Vascular Malperfusion, Evidence of Utero-Placental Separation, Fibrinoid, Intervillous Thrombi and Chronic Inflammation [13]. Based on this consensus statement, a synoptic reporting tool was generated **(Appendix A.1.)** to further promote standardization of placental pathology reporting and disseminations of findings for research and clinical purposes [9].

Placental pathology examinations can provide important clinical information for postpartum maternal and neonate health, evidenced by associations between specific placental lesions and short and life-long adverse health outcomes [9,14-17]. In addition, certain placental lesions are associated with re-occurrence rates that range between 34-100%, providing

important obstetrical information regarding risks for future pregnancies [18]. Placenta pathology can also provide insight to the pathogenesis of various adverse pregnancy outcomes such as placenta-mediated diseases [9,11]. Therefore, placenta pathology is an important clinical tool in understanding disease processes and predicting future adverse health outcomes.

1.2 Preeclampsia

Clinical Description

PE is a hypertensive disorder of pregnancy that occurs in 3-8% of all pregnancies [19]. Globally, it is one of the leading causes of maternal and perinatal mortality and morbidity [20]. Its diagnostic criteria include new on-set hypertension (systolic blood pressure >140 mmHg and diastolic blood pressure >90 mmHg) after 20 weeks' gestation along with maternal end-organ damage including renal, hepatic, neurological, hematological, or utero-placental complications [21]. PE can progress in severity, especially if unmanaged. In certain cases, it can lead to eclampsia (occurrence of a grand mal seizure) or HELLP syndrome (occurrence of hemolysis, elevated liver enzymes and thrombocytopenia) [21]. Current treatment involves management of maternal and fetal symptoms and if indicated, early delivery of the fetus and placenta [21]. As iatrogenic delivery can be harmful for the fetus and prolongation of pregnancy can progress maternal PE, delivery timing must be optimized to balance the risk-benefit ratio [19]. Consequently, PE is highly associated with pre-term delivery, delivery by caesarean section (c-section), admission to neonatal intensive care unit (NICU), and fetal growth restriction [22-24]. The effects of PE can also extend beyond the postpartum period, with the potential for lifelong implications [25]. Notably, there is an increased risk for future cardiovascular disease (CVD) for both mother and child [25,26].

Role of the Placenta

Although its pathogenesis is yet to be fully understood, research strongly supports the involvement of the placenta [19,21,27,28]. This is supported by the improvement in maternal symptoms upon delivery in most cases [27]. As such, PE can also be classified as a placenta-mediated disease [6]. A two-stage mechanism of its pathogenesis is proposed within the literature [19]. The first stage, occurring in the first and early second trimester, involves abnormal placentation [19]. In PE, EVT invasion of the uterine wall is shallow compared to healthy controls [28]. In some cases, invasion was not observed beyond the decidual and myometrial junction [28]. Furthermore, remodelling of uterine spiral arteries into high-capacity, low-resistance vessels to sustain fetal development is incomplete, with smaller vessel diameters compared to normotensive controls [27,29]. This may be a result of disturbances in the EVT differentiation pathway [19,28]. Together, abnormal placentation and insufficient spiral artery remodelling result in poor placental perfusion and hypoxic trophoblast tissue [19]. This is supported by findings of increased lesions of maternal vascular malperfusion and reduced utero-placental blood flow on Doppler ultrasound examinations prior to 20 weeks' gestation in PE cases [27,28,30]. The second stage of this model involves the maternal response to abnormal placentation, with evidence of maternal disease becoming apparent in the latter half of pregnancy. Its main features are maternal systemic endothelial dysfunction and vascular inflammation as evidenced by increased levels of fibronectin, factor VIII antigen and thrombomodulin [19,28]. Placental hypoxia is believed to trigger an oxidative-stress response involving the secretion of anti-angiogenic factors, free radicals, oxidized lipids, and pro-inflammatory cytokines into maternal circulation [19,27]. Two anti-angiogenic factors of note

include soluble FMS-Like Tyrosine Kinase-1 (sFLT-1) and soluble endoglin (sEng). These factors are highly associated with PE as their concentration in maternal circulation correlates with disease progression and decreases upon delivery [31-33]. sFLT-1 binds to vascular endothelial growth factor (VEGF) and placental growth factor (PIGF) decreasing their angiogenic activity on vascular tissues [31]. sENG inhibits transforming growth factor beta (TGF-B) signaling and the downstream vasodilation effects of this pathway on endothelial cells [32,33]. Upregulation of sFLT-1 and sENG are shown to emulate PE symptoms in animal models and are hypothesized to work in unison to cause widespread endothelial dysfunction [32,33]. This dysfunction then results in the clinical presentation of PE- systemic hypertension and end-organ hypoperfusion [19]. Despite support for this model, other theories also exist. Of note, the immunological theory postulates that maternal-fetal allograft rejection contributes to PE pathogenesis [19]. This is supported by increased tumour necrosis factor alpha (TNF-a) and interleukin-6 (IL-6) in PE pregnancies [19]. These factors can cause apoptosis of EVT leading to their decreased proliferation and ability to contribute to sufficient placentation [19]. Decreased levels of human leukocyte antigen (HLA) C, G and E can also impair spiral artery remodelling. In addition, the presence of these inflammatory factors in maternal circulation can further the endothelial dysfunction observed in stage 2 of the previously described model [36]. Further, environmental, genetic, and underlying pre-disposing maternal factors may also contribute to PE pathology [37]. As noted by the spectrum of maternal end-organ dysfunction as well as the variety of potential disease mechanisms, PE is likely a heterogeneous disorder of multiple etiologies [34,35].

Preeclampsia Disease Subclasses

Our research group has furthered the exploration of the heterogenous nature of PE using placental molecular profiling and histopathology to uncover 3 clinically distinct subclasses [34,35]. The first subclass, maternal maladaptation PE, is characterized by normal placental gene expression, appropriate for gestational age infants, normal placental weights, and minimal placental pathology findings [34,35]. The second subclass, hypoxic PE, is characterized by an increased expression of genes related to a hypoxic response (FLT-1, ENG, leptin (LEP) and inhibin subunit beta A (INBA)), more severe maternal end-organ damage, pre-term deliveries, lower placental weights, and placental lesions consistent with decidual vasculopathy and poor utero-placental perfusion of the placenta [34,35]. The third subclass, inflammatory PE, is characterized by increased expression of inflammatory genes related to a pro-inflammatory response, late pre-term deliveries, gradient of fetal growth restriction, evidence of allograft rejection, and placental lesions consistent with chronic inflammation and maternal-fetal interface disturbance [34,35]. In a further immunohistochemistry study, an increase in maternal immune cells identified by cluster of differentiation 68 (CD68) was observed in inflammatory PE cases. Conversely, a decrease in these cells was observed in the hypoxic PE subclass [38]. These results represent an important step in understanding the multifactorial complexity of PE pathogenesis and the potentiality to diagnose and therapeutically target PE subclasses in clinical practice.

1.3 Preeclampsia and Future Cardiovascular Disease

Epidemiology

Epidemiological evidence has well established that women diagnosed with PE are at increased risk for future atherosclerotic cardiovascular disease (CVD) [25]. More specifically, PE women have a 3.7-fold increased risk for chronic hypertension, 4.19-fold for heart failure, 2-fold

for ischemic heart disease, 1.81-fold for stroke, 2.50-fold for coronary artery disease, 1.48-fold for atrial arrythmias, 2-fold for venous thromboembolism, and 2.5-9.5-fold for cardiovascular mortality [25,39]. These effect sizes are comparable to those associated with conventional risk factors such as smoking and diabetes [40]. CVD following PE may also be pre-mature, with the average age of the first cardiovascular event occurring at 38 years, approximately 4-10 years post-PE pregnancy [41].

Potential Mechanisms

Though the relationship between PE and CVD is not completely understood, possible explanations are as follows. Pregnancy may act as a physiological "stress test" due to the maternal organ adaptations occurring throughout pregnancy [42]. Those with underlying CVD risk factors may be less effective at carrying out these adaptations, leading to a disease state manifested by PE [42]. This is supported by the increased presence of traditional risk factors for CVD prior to PE diagnosis including abnormal lipid levels and increased body mass index (BMI) [39,43,44]. Further prospective studies revealed abnormal arterial stiffness and hemodynamics in the first trimester of pregnancy of those that went on to develop PE [45,46]. On the other hand, underlying risk factors for CVD are not present in all women who develop PE [44,47]. Thus, the endothelial dysfunction and hypertension in PE may have lasting impacts on the maternal cardiovascular system and directly contribute to the development of CVD. In some cases, PE is associated with increased risk for pro-atherogenic metabolic syndrome in the immediate postpartum period as observed by increased cardiac output, hypercoagulability and increased inflammatory response [48]. These alterations may be present after conception and have been documented to persist several years postpartum [39,44,49].

1.4 Current Clinical Landscape

Cardiovascular Risk Assessments

PE is now recognized as a sex-specific CVD risk factor by cardiovascular societies worldwide [50]. Their current clinical recommendation is a CVD risk screening assessment for women with a history of PE [50]. These assessments can be completed by a variety of methods, with the most common including indirect 10-year, 30-year, and life-time cardiovascular risk estimation and direct arterial health assessments [50]. CVD risk estimates typically involved risk stratification based on standard clinical and biochemical markers of CVD [51]. On the other hand, arterial health assessments provide further information by directly characterizing arterial stiffness, hemodynamic load, and microvascular endothelial function [52]. Regardless of methods used, by the end of the first-year postpartum, approximately 40% of all PE women who are screened are deemed to be high-risk for lifetime CVD [47]. Furthermore, the majority will go on to develop at least one traditional CVD risk indicator between 1-10 years postpartum [39]. By 2.5-years postpartum, PE women have increased measures of blood pressure, BMI, waist circumference, hemoglobin A1C, total cholesterol, triglycerides, c-reactive protein, and high-density lipoprotein (HDL) compared to normotensive controls [39]. Based, on these findings CVD risk assessments are recommended within the first-year postpartum [47]. In addition, the immediate postpartum period offers an optimal window for risk screening due to the continuous interaction with the healthcare system and potential enhanced motivation for lifestyle changes [47].

Postpartum Screening Clinics

To facilitate CVD risk assessments for this population, specialized post-partum screening clinics have been established worldwide [53]. Currently there are 17 of these clinics in 13 Canadian cities [54]. The first Canadian clinic, the Maternal Health Clinic, was established in 2011 in Kingston, Ontario [53]. Here, CVD risk assessments are performed within 1-year postpartum for those diagnosed with a pregnancy-related CVD risk indicator (including PE) in accordance with the current clinical guidelines from the Canadian Cardiovascular Society [53]. A lifetime cardiovascular risk score is calculated from clinical and biochemical cardiovascular biomarkers obtained from a complete medical history and physical examination [53]. This method of risk estimation has been validated in PE women [51]. A copy of the risk assessment form from this clinic is attached in **Appendix A.2**. Those identified as high-risk are counselled on healthy lifestyle modifications, recommended pharmacotherapy, and referred to further cardiovascular rehabilitation programs if indicated [53].

However, despite these advances, most women with a history of PE do not receive a cardiovascular risk assessment in Canada [54-56]. The following represent current barriers to preventional care for PE women: sheer volume of patients requiring screening, differences in provider knowledge on CVD risk following PE and subsequent referral patterns, low patient adherence, and the absence of a triage prioritization system [54-60]. First, although approximately 20,000-45,000 women are diagnosed with PE annually in Canada, the number of risk assessments to be completed is even greater as recent research has shown that all placenta-mediated diseases are pregnancy-related cardiovascular risk indicators [1,61,62]. Approximately 172,500 women are diagnosed with these disorders annually in Canada [6,61,62]. Second, many providers are unaware of the relationship between PE and CVD as well as the current clinical

guidelines for risk screening for this population [55,57-59]. Knowledge of this relationship was reported at 55% within Canadian primary-care providers [58]. An additional Ontario study indicated that only 20% of women with a hypertensive disorder of pregnancy reported receiving counselling on their risk for future CVD from their care provider [55]. Thus, post-partum clinics that function on a referral basis may miss an important proportion of eligible patients. Third, the non-attendance rates for follow-up appointments are reported between 25-50% at multiple Canadian clinics [54,60,63]. This may be due to lack of time or childcare solutions, especially given the post-partum period [60]. Importantly, non-attendees of the Kingston clinic also reported a decreased risk perception of their future cardiovascular risk [60]. Thus, wide-spread education initiatives for pregnant individuals are warranted to increase awareness on the importance of CVD risk screening after certain pregnancy complications [39,54,59]. Finally, clinics across Canada report finding ~60% of PE patients low-risk for future CVD at the time of screening [44,47,54]. Therefore, risk assessments for all women with PE or other pregnancy related cardiovascular indicators may be unnecessary. Further research into a prioritization system is warranted, especially given the multifactorial nature of these complications. Collectively, these challenges demonstrate the need for a systems-level triage strategy for CVD risk screening and targeted education following PE [54].

1.5 Rationale

This thesis will explore the relationship between unique placental features and findings available at the time of delivery and lifetime maternal risk for CVD. First, a systematic review is presented, summarizing the current state of knowledge and existing knowledge gaps related to the use of cardiovascular risk estimates in the post-partum period for individuals who had a

pregnancy complicated by a placenta-mediated disease. Through a comprehensive analysis of work carried out to date, proposed best practices for post-partum CVD risk screening are discussed. This review is followed by two integrated studies carried out on a joint cohort of individuals who experienced a pregnancy complicated by PE, addressing the association between placental pathology phenotypes (identified via histopathology and immunohistology analysis) and lifetime CVD risk. In the first of these two primary research studies, our main objective is to evaluate the use of placental histopathology as a triage strategy to identify women who are most likely to have a high-risk CVD profile at the time delivery following PE. This strategy would offer screening prioritization to those with placental lesions found to be most associated with high cardiovascular risk estimates in the postpartum period, flagging a group of PE women who would benefit most from referral to postpartum CVD risk screening programs and early intervention. There is great potential for this strategy to be cost-effective, as placental pathology exams are already standard of care for PE pregnancies [12]. As CVD is preventable in ~80% of cases, unique early detection strategies are highly warranted to decrease the future financial and resource burden of CVD on the healthcare system as well as to improve female mortality and morbidly rates [40,53,54]. In the second of the two primary research studies the individual and/or combined association of placental immuno-expression of FLT-1, ENG, and CD68 and lifetime CVD risk profiles is assessed in a subset of the same cohort of PE patients– determining if these may serve as biological markers within the placenta that can be used to identify women at highest risk for future CVD. This will further the understanding of PE heterogeneity through the lens of future maternal health outcomes. Collectively, we will discuss the value of exploiting unique placental features and findings in predicting future cardiovascular risk, by bringing together the

risk profiles of placenta-mediated diseases, associations between placental pathology findings and cardiovascular risk following PE and associations between placental immune-expression profiles and cardiovascular risk following PE.

1.6 Hypothesis

The overarching hypothesis of this MSc thesis is that detailed assessment of the placenta following delivery can contribute to the identification of women at high lifetime -risk for CVD following a diagnosis of PE via the use of placental histopathology and immuno-expression of FLT-1, ENG, and CD68. We hypothesize that PE subclasses will have differing CVD risk profiles revealed through differences in placental phenotypes. A graphical explanation of these relationships is presented in **Figure 1.2**.

1.7 Research Aims

Research Aim 1: To perform a systematic review of the literature to determine the estimated risk of CVD associated with a diagnosis of placenta-mediated disease during pregnancy.

Sub-aim 1: To examine the presence of traditional cardiovascular risk factors following placenta-mediated diseases of pregnancy.

Sub-aim 2: To offer recommendations for CVD screening protocols for this population.

Research Aim 2: To investigate the association between placental histopathology lesions and estimated lifetime cardiovascular risk following PE.

Sub-aim 1: To determine the association between individual placental histopathology lesions and lesion categories with elevated lifetime cardiovascular risk at 6 months postpartum.

Sub-aim 2: To develop a predictive model for elevated lifetime cardiovascular risk based on placental histopathology and clinical findings.

Research Aim 3: To examine the potential relationship between PE subclass and estimated cardiovascular risk via immunohistochemistry analysis of placental specimens.

Sub-aim 1: To compare the relative expression of FLT-1, ENG, and CD68 in placental samples with PE and healthy controls.

Sub-aim 2: To compare the relative expression of FLT-1, ENG, and CD68 by cardiovascular risk score.

Sub-aim 3: To further evolve the predictive model for elevated cardiovascular risk based on placental histopathology and clinical findings with the additional inclusion of placenta protein expression of FLT-1, ENG and CD68.

In this thesis, a series of manuscripts are presented which address each of the research aims and/or sub-aims.

1.8 Tables and Figures

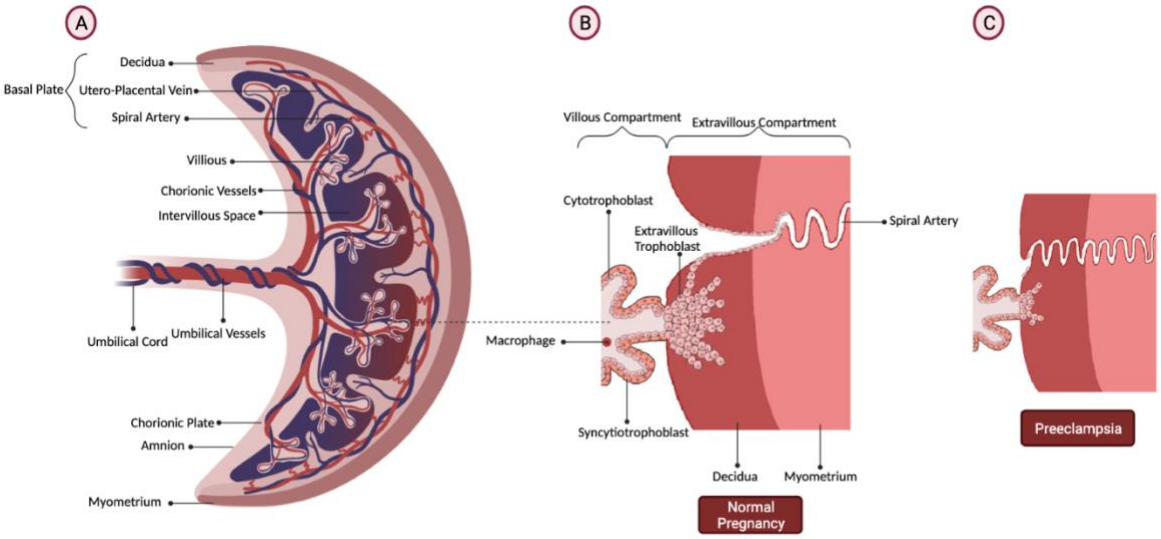

Figure 1.1. Schematic Representation of the Human Placenta. (A) Cross-section of human placental structure. The umbilical cord and chorionic villi originate from the fetal chorionic plate. The basal plate is composed of decidua and maternal blood vessels. The intervillous space is found between the two plates and contains maternal blood, which bathes the chorionic villous structures. **(B-C)** Cross section of the two functional compartments of the placenta. **(B)** The villous compartment contains the chorionic villi – sites of maternal-fetal exchange – which are encased in the multinucleated syncytiotrophoblasts cellular layer. These cells are formed via terminal differentiation of the underlying layer of villous cytotrophoblasts. Encased within the villi are fetal capillaries embedded in a mesenchymal core that harbours fibroblasts and immune cells such as macrophages. The extra-villous component contains the extra-villous trophoblasts (EVT), also derived from cytotrophoblasts, which invade the decidua and inner myometrium and remodel the uterine spiral arteries into large diameter vessels. **(C)** Placentation in preeclampsia. Compared to normal pregnancies, extra-villous trophoblast invasion is shallow and spiral artery remodelling is incomplete. Created with BioRender.com

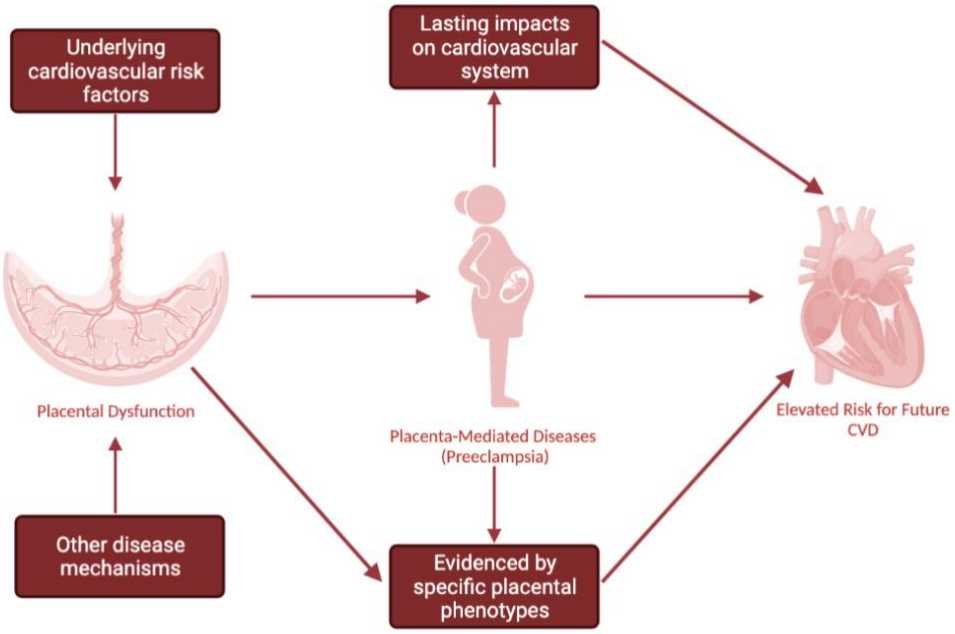

Figure 1.2. Graphical Illustration of Study Hypothesis. Placental dysfunction during pregnancy may be caused by underlying cardiovascular risk factors or other disease mechanisms, identifying those with sub-clinical risk for future CVD. This can lead to the development of a placenta-mediated disease such as PE. This disease state can have lasting impacts on the cardiovascular system and lead to elevated risk for future CVD. Differing placental phenotypes assessed by histopathology and immunohistochemistry findings may be able to predict elevated estimated risk for CVD, identifying a subclass of PE women who would benefit most from referral to postpartum CVD screening clinics and early intervention. Created with BioRender.com.

Chapter 2

A systematic review of the association between placenta-mediated diseases during pregnancy and estimated maternal risk for cardiovascular disease

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Abstract

Objective: Assess the epidemiological association between history of placenta-mediated diseases (PMD) (exposure of interest) and maternal cardiovascular disease (CVD) risk factors in the post-partum period (outcome of interest).

Background: PMDs encompass pregnancy complications resulting from abnormal placental development and/or function. In recent years, multiple PMDs have been associated with future CVD and are recommended for additional screening for CVD risk in the postpartum period. However, as implementation of these recommendations is still in its infancy, improvements to risk screening strategies are warranted.

Inclusion Criteria: Inclusion criteria included peer-reviewed observational studies that measured maternal cardiovascular risk factors in the postpartum period (assessed using biochemical and clinical CVD risk markers) following PMDs of pregnancy. Our exclusion criteria included book chapters, commentaries, conference abstracts, systematic reviews, cases studies, interventional studies, studies including hypertensive disorders of pregnancy collectively, studies assessing low-birth weight (in the absence of confirmed placental disease), studies measuring outcomes of interest prior to or during pregnancy, studies that assessed fetal outcomes, and studies that directly measured CVD morbidity and mortality.

Methods: MEDLINE (Ovid), EMBASE (Ovid), CINAHL (EBSCOhost), and Scopus databases were searched from inception to June 27, 2022 for observational studies on the estimated risk for future CVD following a PMD – including: preeclampsia, HELLP syndrome, fetal growth restriction, small for gestational age, placental abruption, pre-term birth, gestational diabetes, or stillbirth.

The search was limited to articles in English or French. Risk of bias was assessed by the Newcastle-Ottawa quality assessment tool. Results are presented in a narrative and tabular summary.

Results: The search yielded 11,039 articles of which 104 met our inclusion criteria. The majority of included studies were cohort studies. Sample sizes ranged from 21 – 116,429 participants and CVD risk factor assessment timing ranged from 1.44 months to 35 years postpartum. The majority of the studies evaluated preeclampsia and gestational diabetes while a smaller proportion evaluated preterm birth, stillbirth, and placental abruption independently. All PMD types demonstrated evidence of increased CVD risk markers at varying timepoints postpartum. At least one study per PMD type had non-optimal measures of systolic blood pressure, BMI, and total cholesterol. PE studies demonstrated an increase in systolic blood pressure and BMI over time.

Conclusions: Women with a history of PMDs demonstrate heightened evidence of CVD risk markers post-partum. As such, a detailed obstetric history at CVD risk assessments is warranted. Further, the use of elevations in systolic blood pressure, BMI, current smoking status, and total cholesterol may be CVD risk markers specifically relevant to this population of reproductive-age women. Improved risk assessment tools and triage strategies in the postpartum period should be explored for women with a history of PMDs. Future studies should focus specifically on characterizing the CVD risk profiles associated with preterm birth, stillbirth, and placental abruption.

Registration: Protocol is registered on PROSPERO (number: CRD42022341291).

Keywords: Placenta-mediated Diseases; Preeclampsia; HELLP Syndrome; Preterm Birth; Stillbirth; Fetal Growth Restriction; Placental Abruption; Gestational Diabetes; Cardiovascular Disease; Cardiovascular Risk; Systematic Review

1. Introduction

A healthy placenta is key to a healthy pregnancy [1]. Placenta-mediated diseases [PMD] describe a group of pregnancy complications resulting from abnormal placental development and/or dysfunction [2-6]. PMDs can include preeclampsia, eclampsia, Hemolysis Elevated Liver Enzymes and Low Platelets (HELLP) syndrome, fetal growth restriction, placental abruption, stillbirth, and spontaneous preterm birth [2-6]. Additionally metabolic complications such as gestational diabetes mellitus have origins in the placenta, related to abnormal glucose homeostasis and insulin resistance secondary to placental lactogen release [2-6]. Although difficult to estimate, PMDs collectively affect approximately 30% of all pregnancies [2]. While the etiology and pathophysiology of these placental complications differ, there is considerable overlap in the mechanisms underlying these conditions, notably poor utero-placental perfusion and heightened inflammation at the maternal-fetal interface. [2,4]. While short and long-term adverse health outcomes of the complications for both mother and child differ across the spectrum of PMD, PMDs are known to associate with an increased risk for future maternal cardiovascular disease (CVD) [8,9]. More specifically, the pooled odds ratio for CVD-related mortality is 2.5-9.5 for preeclampsia, 1.93 [1.83-2.03] for preterm birth, and 2.23 [1.90-2.62] for stillbirth [8,10]. The odds ratio for CVD-related morbidity and mortality is 1.82 [1.42-2.33] for placental abruption, 1.68 [1.11-2.52] for gestational diabetes, and 1.29 [0.91-1.83] for low birth weight [8]. As cardiovascular disease is a leading cause of death for women, unique sex-specific strategies for risk screening and prevention after pregnancy are warranted [11-13].

Given the strong association between PMD pregnancy complications and future CVD, a postpartum cardiovascular risk assessment is currently recommended for women with a history

of preeclampsia, HELLP, gestational diabetes, fetal growth restriction, and/or preterm birth [9]. This assessment typically involves a patient history and measurements of blood pressure, lipids, and fasting glucose to calculate a 10-year, 30-year, or lifetime cardiovascular risk score [9]. Other methods of evaluating risk for CVD include arterial hemodynamic assessments and vascular Doppler sonography [14,15]. Although current recommendations for additional screening do not include placental abruption or stillbirth, several specialized postpartum screening clinics include these patients due to the shared etiological and pathophysiological mechanisms common to placental complications [8,9,13]. Currently, there is no clear protocol for postpartum cardiovascular risk assessments following PMDs. In addition, it is unknown whether all forms of PMD are at increased risk for CVD. To address these knowledge gaps, this systematic review summarizes the current state of research regarding postpartum CVD risk screening as well as describes the cardiovascular risk profiles following different types of PMDs during pregnancy. Based on our findings, we report any possible recommendations for CVD screening protocols and future research for this population. We hypothesize that due to the shared placental disease mechanisms, all form of PMD will demonstrate evidence of non-optimal cardiovascular health in the postpartum period.

2 Methods

Review Protocol

A preliminary search for systematic reviews on the topic was conducted a priori (PROSPERO, PubMed, and Cochrane Library). Only reviews focusing on CVD morbidity and mortality following PMD were found in addition to one focusing on estimated risk for CVD strictly following a pregnancy complicated by preeclampsia [8]. Hence, work on this systematic review was carried

out to specifically fill the gap in the literature – looking more broadly at summarizing current knowledge on postpartum CVD risk estimation associated with the umbrella of PMDs. The objectives, inclusion criteria, methods of analysis for this review were specified in advance and documented a priori as a protocol registered with PROSPERO (number: CRD42022341291) [16].

Search Strategy

The MEDLINE (Ovid), EMBASE (Ovid), CINAHL (EBSCOhost), and Scopus databases were searched from inception to June 27, 2022. The scope of the search was broad to capture studies that evaluate cardiovascular risk parameters in women with a history of PMDs. The search was limited to articles in French and English, and studies focusing on animals, outcomes in the offspring, and other systematic reviews were excluded. The Medline search strategy including concepts, keywords, syntax and summation used can be found in **Table 2.1**, while comparable search strategies of the other databases can be found in the supplementary material (**Tables S3-S5**). All identified articles were uploaded to the systematic review software, Covidence [17]. Duplicates were removed, abstract and full-text screening of each article were processed by 2 of 3 independent reviewers (EM, ECC, HP), and disagreements were settled by discussion.

Eligibility Criteria

Peer-reviewed observational studies, including cohort, cross-sectional and case-control studies examining maternal CVD risk assessment in women with a history of PMDs were included. For the purposes of this review, PMDs were defined to include: preeclampsia, eclampsia, HELLP syndrome, fetal growth restriction, small for gestational age, placental abruption, preterm birth, gestational diabetes, or stillbirth. Included studies assessed biochemical [total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), triglycerides, and fasting glucose] or

clinical markers [systolic and diastolic blood pressure, current smoking, waist circumference and body mass index (BMI)] for CVD risk estimation or used a combination of these measures to calculate an estimated CVD risk score (5-year, 10-year, 30-year, or lifetime). Randomized control trials, case studies, animal studies, book chapters, conference abstracts, editorials, and studies deficient in primary data were excluded. Studies with a sample size <10, studies of cardiovascular outcomes of offspring, studies directly measuring CVD mortality and morbidity, studies measuring other cardiovascular risk parameters (e.g., hemodynamics, endothelial dysfunction, arterial stiffness), and studies that included participants with chronic pre-existing conditions were also excluded.

Quality Assessment

The quality of each article was assessed by 2 of 3 independent reviewers (EM, ECC, and HP) using the Newcastle-Ottawa quality assessment tool for cohort and case-control studies [18]. We modified the tool to accommodate for cross-sectional studies using the suggestions from Herzog et al [19]. In addition, to offer further specificity, we adapted the following items to our review; item 3: demonstration that the outcome was not already present in the sample was defined as excluding participants with previous CVD or chronic pre-existing conditions; item 6: comparability between controls and cases was defined by controlling for any potentially confounding variables or matching participants by maternal age, gestational age, and/or BMI; item 8: adequacy of follow-up was defined as participation of >80%; item 9: adequate period of time for follow-up to allow outcome to occur was defined as 6 months postpartum [13]; item 10 and 11: adequate ascertainment of outcome was defined as performing a blinded CVD risk assessment. The quality assessment template was uploaded and utilized in Covidence [17]. The template is included in

the supplementary material (**S6**). Any disagreements were settled by discussion. A total score out of 18 was calculated for each study, where high quality was defined 12 and above, medium as between 7-12, and low as below 7 [18].

Data Extraction

Data was extracted by 2 of 3 independent reviewers (EM, ECC, and HP) for each study using a standardized data collection template in Covidence [17]. Any discrepancies in extracted data were discussed and appropriately revised to remove any errors. The following data variables were extracted from all studies: study location, study aim, study design, study dates, inclusion and exclusion criteria, types of PMD included, total sample size, and number of PMD cases and controls. The following was recorded when present for all included PMD cases: average maternal age, gestational age, pre-pregnancy and/or postpartum BMI, waist circumference, placenta weight, birth weight, current smoking, ethnicity, risk assessment timing (years postpartum), blood pressure, fasting glucose level, total cholesterol level, HDL level, LDL level, triglyceride level, methods of CVD risk estimation used, CVD risk screening score, and proportion, relative risk or odds ratio of screening high-risk for CVD. Studies that did not provide all the variables were included and any missing variables were labelled as N/A and were not included in the result section for that specific variable. Any information that was unclear was discussed among the reviewers and a decision was made to include or exclude the variable in question.

Synthesis Methods

Extracted data was summarized by tabular, graphical, and narrative summaries to provide an overview of the studies including study design, available data (assessed biochemical and clinical markers of CVD risk and/or calculated CVD risk score), sample size, and postpartum risk

assessment timing by PMD type. Data extracted from each individual study can be found in supplemental material (**S1**). Scatter plots were created to visualise each individual biochemical and clinical marker of CVD risk assessed by PMD type in increasing order of years postpartum. For each marker assessed, a non-optimal threshold was determined based on published cut-offs [20] and the number of studies whose average fell over this threshold was calculated. A narrative description of CVD risk score data is presented as the initially planned forest plot was difficult to generate due to the high degree of variability between the studies.

Table 2.1 Representative database search strategy using Medline syntax

Concept #	**Search Terms**
Cardiovascular Disease	
1	exp Cardiovascular System/ or exp Cardiovascular Diseases/ or exp Cardiology/ or exp Heart Disease Risk Factors/ or exp Vascular Diseases/ or exp Aortic Diseases/ or exp Cerebrovascular Disorders or exp Cardiomyopathy/
2	(cardiac* or heart* or cardiolog* or cardiovasc* or coronary or vascular* or cerebrovasc* or arterial* or artery or arteries or aorta or aortic or vein* or venous* or valve or valvular or ventric* or arrhythm* or cardiomyopath* or infarct* or stroke* or hypertens* or endocardi* or myocardi* or pericardi* or angiog* or angioc* or Electrocardiograph* or Electrocardiogram* or ECG* or EKG* or holter* or CVD*).ti,ab.
Pregnant Population	
4	exp Pregnancy/ or Pregnant Women/ or exp Obstetrics/ or exp Labor, Obstetric/
5	(pregnan* or obstetric* or postpartum* or postnatal*or (post adj natal*) or (post adj partum*) or (labor adj3 delivery) or labor* or deliver* or maternal*).ti,ab.
Placental Diseases	
6	exp Placenta Diseases/ or exp Pregnancy Complications/ or exp Obstetric Labor Complications
7	(pregnanc* adj3 complicat* or placenta mediated disease* or placenta* adj disease* or pregnanc* adj3 disease* or placenta* adj disorder or labor complicat* or obstetric* adj3 complicat*).ti,ab.
8	exp diabetes, gestational/
9	gestational diabetes.ti,ab.
10	exp Hypertension, Pregnancy-Induced/
11	(eclamp* or pre eclamp* or preeclamp* or pre-eclamp* or hellp syndrome* or hellp* or tox?emi*).ti,ab.
12	Abruptio Placentae/
13	(placent* adj2 abruption).ti,ab.
14	exp Infant, Low Birth Weight/
15	Fetal Growth Retardation/
16	(intrauterine growth restriction* or intra-uterine growth restriction* or IUGR* Or FGR* or low birth weight or small for gestational age ((fetal or foetal or fetus or foetus) adj3 (restriction* or retard*))).ti,ab.
17	exp Fetal Death/
18	(IUFD* or intrauterine f?etal demise or intrauterine neonatal death* or stillbirth* or still birth* or stillborn* or still born* or ((fetal or foetal or fetus or foetus) adj3 (death*))).ti,ab.
19	exp Infant, Premature/
20	(prematur* or preterm* or pre term*).ti,ab.
Study Methodology	
21	exp Cohort Studies/ or exp Case Control Studies or exp Risk Factors/
22	(risk factor* or risk assessment or risk assess* or risk equation or risk calc* or risk scor* or risk predict* or risk factor calc* or prediction model* or scoring* method* or scoring* scheme* or risk estim*).ti,ab.
23	(framingham equation* or framingham estim* or framingham algorithm or framingham guideline* or framingham risk or framingham score or framingham model* or score risk* or score algorithm* or arterial assess* or arterial health assess* or

	hemodynamic* or arterial tonometry or arterial stiffness or hemodynamic load* or vascular doppler* or vascular doppler sonography).ti,ab.
Summation	
21	1 or 2
22	4 or 5
23	or/6-20
24	21 and (22 or 23)
25	21 and 22 and 23 and 24
26	25 not (exp Animals/ Not Humans.sh.)
27	26 not (exp Systematic Review/ or exp Meta-Analysis/ or exp Review/)
28	27 not (child* or offspring*).ti,ab,kw.
29	28 not (exp child/ or adult children/ or exp male/)
30	limit 29 to (english language or french)

3 Results

Article Selection

Our initial search yielded 11,039 articles of which 3,448 were duplicates and removed **(Figure 2.1).** Another 7,243 studies were removed following title and abstract screening and 240 removed following full-text review based on our pre-defined inclusion and exclusion criteria **(Figure 2.1)**. Weighted kappa scores between reviewers for title and abstract screening were deemed as moderate: 0.46 between E.E.M and E.C.C, 0.56 between E.C.C and H.P, and 0.45 between E.E.M and H.P. 462 articles were assessed for discrepancies between authors. A total of 104 articles were included in the data extraction phase our study and are presented in the supplementary material (**S2)**. 52 articles included data on measures of CVD risk assessment, specifically following preeclampsia, eclampsia, or HELLP syndrome; 30 specifically following gestational diabetes; 6 specifically following preterm birth; 2 specifically following fetal growth restriction or small for gestational age; 1 specifically following placental abruption, and 0 specifically following stillbirth. 13 articles examined multiple PMDs, either independently or as a combined group. Of these 13 articles, 10 studies independently measuring CVD risk factors following preeclampsia were found; 4 following gestational diabetes; 3 following preterm birth;

8 following fetal growth restriction or small for gestational age; and 2 following stillbirth. 3 articles assessed multiple PMDs as a combined group.

Study Characteristics

Table 2.2 summarises the characteristics of the included studies. The most common types of studies identified were cohort studies (n=56), followed by cross-sectional (n=38), and case-controls (n=10). Studies were included from around the world, the most recurrent countries included The Netherlands (n=20), The United States of America (n=18), and Canada (n=14). The sample size of the included studies ranged from 21 – 116,429 participants, and the majority of included studies had exclusively or >90% Caucasian participants. All the included studies measured clinical and biochemical markers to assess CVD risk, while only 26 studies went on to calculate a CVD risk score. The timing of the risk assessments of the included studies ranged from 1.44 months to 35 years postpartum with an average of 6.6 years postpartum.

Quality Assessment

A summary of the quality assessment results using the Newcastle-Ottawa tool are presented in a supplementary table (**S1**). 57 studies were deemed high quality, 45 deemed medium quality, and 2 deemed low quality. Studies with the highest quality assessment score were those assessing CVD risk following a pregnancy complicated by gestational diabetes or preeclampsia, whereas the lower quality assessment scores were most often found in studies that assessed CVD risk following multiple PMDs. The two low quality studies pertained to preeclampsia and small for gestational age. These studies particularly lacked the inclusion of controls, did not exclude participants with chronic co-morbid conditions, lacked adequate of follow-up, did not provide sample size justification, and lacked representativeness of the

participants. Some of the main areas of weaknesses across all studies were the lack of adjustment for confounders, self-report of PMD history, and insufficient sample sizes.

Clinical Markers of Cardiovascular Risk

Figures 2.2 and 2.3 present the average postpartum blood pressure measures by PMD type for each relevant study. A slight trend of increasing systolic blood pressure in the postpartum period over time was found for preeclampsia (from 0.14-35 years postpartum) and gestational diabetes (from 0.12-20 years postpartum). Higher measures of postpartum blood pressure were found in the preeclampsia studies compared to the other types of PMDs assessed (Highest study average recorded (systolic/diastolic): 147/99 mmHg for preeclampsia vs 135/80 mmHg for gestational diabetes, 128/84 mmHg for fetal growth restriction/small for gestational age, 128/78 mmHg for preterm birth, 122/77 mmHg for stillbirth, 121/80 mmHg for placental abruption and 120/78 mmHg for multiple PMDs). Moreover, 49 out of 103 studies (50%) reported non-optimal average systolic blood pressures (above 120 mmHg) according to the lifetime CVD risk estimate threshold [20]. More specifically, 33 out of 57 preeclampsia studies were above this threshold (58%), 9 out of 25 for gestational diabetes (36%), 3 out of 8 for fetal growth restriction/small for gestational age (38%), 2 out of 8 for preterm birth (25%), 1 out of 1 for stillbirth (100%), 1 out of 1 for placental abruption (100%), and 1 out of 3 for multiple PMDs (33%). 24 out of 98 studies reported non-optimal average diastolic blood pressures (above 80 mmHg) according to the lifetime CVD risk estimate threshold [20]. 21 out of 55 preeclampsia studies were above this threshold (38%), 1 out of 25 for gestational diabetes (4%), 0 out of 7 for fetal growth restriction/small for gestational age (0%), 1 out of 6 for preterm birth (17%), 0 out of 1 for stillbirth (0%), 0 out of 1 for placental abruption (0%), and 0 out of 3 for multiple PMDs (0%).

Figure 2.4 presents postpartum BMI measurements by PMD type for each relevant study. BMI measurements were included in 108 studies, of which 8 reported pre-pregnancy BMI only. A slight trend in increasing BMI indices over time in the postpartum period was observed in GDM studies (from 0.12-20 years postpartum). The highest study averages for postpartum BMI recorded was 32.3 kg/m^2 for preeclampsia, 32.1 kg/m^2 for gestational diabetes, 31.4 kg/m^2 for fetal growth restriction/small for gestational age, 29.9 kg/m^2 for preterm birth, 32.3 kg/m^2 for stillbirth, 25.7 kg/m^2 for placental abruption, and 25.9 kg/m^2 for multiple PMDs. 86 studies out of 108 (79.6%) reported average postpartum BMIs in the overweight range (above 25 kg/m^2) according to threshold used in the 10-year CVD risk estimate [20]. 48 out of 58 preeclampsia studies were above this threshold (82.8%), 26 out of 31 for gestational diabetes (84%), 4 out of 8 for fetal growth restriction/small for gestational age (50%), 4 out of 6 for preterm birth (68%), 1 out of 1 for stillbirth (100%), 1 out of 1 for placental abruption (100%), and 2 out of 3 for multiple PMDs (68%). Regarding studies who reported pre-pregnancy BMI, 19 measurements were found. 11 of these were considered overweight (above 25 kg/m^2).

Figure 2.5 presents postpartum waist circumference by PMD type for each relevant study. 48 measurements of waist circumference were recorded. The highest study averages for postpartum waist circumference were 102.7 cm for preeclampsia, 102.3 cm for gestational diabetes, 91.7 cm for preterm birth. No measures of waist circumference were reported for fetal growth restriction/small for gestational age, stillbirth, or placental abruption. 27 studies out of 48 (56%) reported waist circumference measures above the non-optimal threshold of 88cm in accordance with the 10-year CVD risk assessment threshold [20]. 9 out of 23 preeclampsia studies

were above this threshold (39%), 15 out of 23 for gestational diabetes (65%), and 3 out of 3 for preterm birth (100%).

Figure 2.11 presents current smoking status by PMD type for each relevant study. The highest rates of current smoking within study samples were 49% for preeclampsia, 47% for gestational diabetes, 45% for fetal growth restriction/small for gestational age, 57% for preterm birth, 21% for stillbirth, 24% for placental abruption, and 18% for multiple PMDs. According to lifetime CVD risk estimates, any current smoking is non-optimal [20].

Biochemical Markers of Cardiovascular Risk

Figure 2.6 presents average postpartum cholesterol levels by PMD type for all relevant studying. 98 measures of cholesterol levels were reported. A slight trend in increasing cholesterol levels over time in the postpartum period was observed in preeclampsia studies (from 0.14-35 years postpartum). The highest study averages for cholesterol levels were 6.64 mmol/L for preeclampsia, 5.5 mmol/L for gestational diabetes, 5.7 mmol/L for fetal growth restriction/small for gestational age, 5.7 mmol/L for preterm birth, 5.01 mmol/L for stillbirth, 5.18 mmol/L for placental abruption, and 4.8 mmol/L for multiple PMDs. Further, 79 studies out of 98 (81%) reported non-optimal average cholesterol levels (above 4.65 mmol/L) according to the lifetime CVD risk estimate [20]. 41 out of 52 preeclampsia studies were above this threshold (79%), 24 out of 28 for gestational diabetes (86%), 5 out of 6 for fetal growth restriction/small for gestational age (83%), 5 out of 6 for preterm birth (83%), 1 out of 1 for stillbirth (100%), 1 out of 1 for placental abruption (100%), and 2 out of 2 for multiple PMDs (100%).

Figure 2.7 presents HDL measurements by PMD type for relevant studies. 108 measures of HDL levels were reported. The highest study averages for HDL levels were 1.96 mmol/L for

preeclampsia, 1.74 mmol/L for gestational diabetes, 1.58 mmol/L for fetal growth restriction/small for gestational age, 1.61 mmol/L for preterm birth, 1.45 mmol/L for stillbirth, 1.3 mmol/L for placental abruption, and 1.5 mmol/L for multiple PMDs. 26 out of 108 (24%) studies reported non-optimal average HDL levels (less than 1.3 mmol/L) according to threshold in the lifetime CVD risk estimate [20]. Average HDL levels in 13 out of 57 preeclampsia studies were below this threshold (23%), 8 out of 30 for gestational diabetes (27%), 3 out of 8 for fetal growth restriction/small for gestational age (38%), 0 out of 7 for preterm birth (0%), 0 out of 2 for stillbirth (0%), 0 out of 1 for placental abruption (0%), and 2 out of 3 for multiple PMDs (68%).

Figure 2.8 presents LDL measurements by PMD type for relevant studies. 94 measures of LDL levels were reported. The highest study averages for LDL were 3.85 mmol/L for preeclampsia, 4.16 mmol/L for gestational diabetes, 3.34 mmol/L for fetal growth restriction/small for gestational age, 3.34 mmol/L for preterm birth, 3.13 mmol/L for stillbirth, 3.19 mmol/L for placental abruption, and 2.90 mmol/L for multiple PMDs. 5 out of 94 (5%) studies reported non-optimal average LDL levels (above 3.5 mmol/L) according to the 10-year CVD risk estimate [20]. 4 out of 47 preeclampsia studies reported average LDL levels above this threshold (6%), 1 out of 30 for gestational diabetes, (3%), 0 out of 7 for fetal growth restriction/small for gestational age, 0 out of 5 for preterm birth (0%), 0 out of 2 for stillbirth (0%), 0 out of 1 for placental abruption (0%), and 0 out of 2 for multiple PMDs (0%).

Figure 2.9 presents triglyceride measurements by PMD type for relevant studies. 103 measures of triglyceride levels were reported. The highest average levels were 3.6 mmol/L for preeclampsia, 2 mmol/L for gestational diabetes, 1.2 mmol/L or fetal growth restriction/small for gestational age, 1.32 mmol/L for preterm birth, 1.2 mmol/L for stillbirth, 1.64 mmol/L for

abruption, and 0.9 mmol/L for multiple PMDs. 7 out of 103 (7%) studies reported non-optimal average triglyceride levels (above 1.17 mmol/L) according to the lifetime CVD risk estimate [20] with 6 out of 51 preeclampsia studies above this threshold (12%) and 1 out of 31 for gestational diabetes (3%). Triglyceride levels were below the non-optimal threshold for studies reporting fetal growth restriction, preterm birth, stillbirth, placental abruption and combined PMDs.

Figure 2.10 presents fasting glucose measurements by PMD type for relevant studies. 81 measures of glucose levels were reported. The highest reported study average levels were 6.32 mmol/L for preeclampsia, 6.44 mmol/L for gestational diabetes, 5.12 mmol/L for fetal growth restriction/small for gestational age, 5.09 mmol/L for preterm birth, 5.1 mmol/L for placental abruption, and 5.5 mmol/L for multiple PMDs. 15 out of 81 (18%) studies reported non-optimal fasting glucose levels (above 5.6 mmol/L) according to the lifetime CVD risk estimate [20]. 5 out of 47 preeclampsia studies were above this threshold (11%), 10 out of 24 for gestational diabetes (42%), 0 out of 5 for fetal growth restriction/small for gestational age (0%), 0 out of 1 for preterm birth (0%), 0 out of 1 for placental abruption (0%), and 0 out of 3 for multiple PMDs (0%). No

Cardiovascular Risk Estimates

26 studies went on to calculate CVD risk scores by a variety of methods. The most common method was the 10-year risk estimate. Only 5 measurements of lifetime CVD risk were reported. Of these 26 studies, only 7 reported odd ratios of elevated CVD risk following gestational diabetes (n=2), preeclampsia (n=3), and preterm birth (n=2). All but one of these studies reported significant odds ratios. The insignificant finding was a study of gestational diabetes. The remaining studies that evaluated CVD risk estimates reported raw scores only. For gestational diabetes, an odds ratio for elevated CVD risk of 3.01 [2.11, 3.72] was reported. For preeclampsia,

odds ratios for elevated CVD risk of 1.47 [1.071, 2.019; 0.031], 2.34 [1.03, 5.59; 0.045], and 2.5 [p-value= 0.001] were reported. For preterm birth, odds ratios for elevated CVD risk of 1.74 [1.28, 2.37] and 2.31 [1.82, 2.87] were reported.

4 Discussion

Placenta-Mediated Diseases and Cardiovascular Risk

This comprehensive systematic review of 104 studies presents a novel summary of the estimated postpartum CVD risk profiles following 6 different PMDs of pregnancy. Collectively, we illustrated that all types of PMDs demonstrate heightened presence of risk factors for future CVD as detected by clinical and biochemical markers. Studies with multiple PMDs demonstrated evidence of non-optimal systolic blood pressure (n=1; 33.3%), BMI (n=2; 67.7%), total cholesterol (n=2; 100%), and HDL (n=2; 67.7%). Evidence of hypertensive systolic blood pressures (above 120 mmHg); BMIs in the overweight range (above 25 kg/m^2); any current smoking (above 0%), and non-optimal elevations in total cholesterol (above 4.65 mmol/L) were reported in at least one study for all types of PMDs [20]. These findings correlate with other epidemiological-based systematic reviews where forms of PMDs are associated with an increased incidence of hypertension and obesity [8]. Further, these markers could be explored in a unique CVD risk estimation model specific to younger women of reproductive age with a history of PMDs. Currently, these measures are included in universal 10-year and lifetime risk estimates [20]. Total cholesterol is used in universal lifetime CVD risk estimates, the current preferred method of estimating CVD risk in women with a history of PE while BMI is used in 10-year CVD risk estimates [20]. Systolic blood pressure is used in both methods of risk estimation [20].

Preeclampsia

As preeclampsia represents the first PMD to be classified as a pregnancy-related CVD risk indicator, it unsurprisingly represented the majority (50%) of studies in our review [21]. Preeclamspia studies demonstrated non-optimal evidence of all CVD risk measures assessed: systolic blood pressure (n=33; 58.9%), diastolic blood pressure (n=22; 40.7%), BMI (n=48; 82.8%), total cholesterol (n=41; 78.8%), HDL (n=13; 22.8%), LDL (n=4; 6.4%), triglycerides (n=6; 11.8%), and glucose (n=5; 10.6%). The large proportion of preeclampsia studies allowed us to evaluate temporal trends in CVD risk parameters from 0.12-35 years postpartum. Interestingly, we observed a slight increasing trend in systolic blood pressure and total cholesterol levels over time. These findings are in line with other reports of both short and long-term cardiovascular dysfunction women with a history of preeclampsia [10]. Further, this dysfunction has been reported to worsen over time in longitudinal studies [10].

Gestational Diabetes

Gestational diabetes represented the second most common PMD type in our review. Gestational diabetes studies demonstrated non-optimal evidence of all CVD risk measures assessed: systolic blood pressures (n=10; 40%), diastolic blood pressures (n=1; 4%), BMI (n=26; 23.9%), total cholesterol (n=24; 85.7%), HDL (n=8; 26.7%), LDL (n=1; 3.3%), triglycerides (n=1; 3.2%), and glucose (n=10; 41.7%). As such, we also observed an increasing trend in postpartum systolic blood pressure over time. This may indicate that the incidence of hypertension following gestational diabetes may increase with age.

Fetal Growth Restriction/Small for Gestational Age

Fetal growth restriction/small for gestational age studies demonstrated evidence of non-optimal systolic blood pressure (n=3; 37.5%), BMI (n=4; 50%), total cholesterol (n=5; 83.3%), and HDL (n=3; 37.5%). Due to a low proportion of studies evaluating these PMD (n=10), we presented fetal growth restriction and small for gestational age results together. Considering the overlapping nature of placental disease in cases of fetal growth restriction/small for gestational age and preeclampsia [2], and the small volume of data collected on postpartum CVD risk, further research on postpartum risk profiles in this patient population is warranted.

Preterm Birth

An even smaller proportion of studies evaluated CVD risk profiles in the postpartum period following a preterm birth (n=9). Preterm birth studies demonstrated evidence of non-optimal systolic blood pressure (n=2; 25%), diastolic blood pressure (n=1; 16.7%), BMI (n=4; 66.7%), and total cholesterol (n=5; 83.3%). It is likely that preterm birth was a common co-morbidity in our included studies, evidence by only 6 studies with average gestational age at delivery above the preterm range (>37 weeks). It should be noted that preterm birth as a co-morbidity to other PMDs was often not clearly identified or adequately discussed in the interpretation of study results. Given the considerable prevalence of preterm birth across the globe, the associations between preterm birth (with and without evidence of other PMDs) and CVD risk markers in the postpartum period certainly requires more in-depth investigation [22].

Stillbirth

Examinations of CVD risk markers exclusively following a stillbirth were underrepresented in the literature, with only 2 studies specifically examining these relationships. These studies,

however, did demonstrated evidence of non-optimal systolic blood pressure (n=1; 100%), BMI (n=1; 100%), and total cholesterol (n=1; 100%) in the postpartum period These findings are in line with a morbidity-based systematic review where women with a history of stillbirth also presented evidence of increased risk for CVD and were 49% more likely to experience a CVD event [8]. An additional 7 studies reported co-morbid stillbirth proportions ranging from 5.9-67%. These studies also presented evidence of non-optimal systolic blood pressure (Bokslag 2017; PE w/ 13.7% stillbirth, Drost 2012; PE w/ 14.8% stillbirth, Ernawati 2019; PE w/ 5.9% stillbirth), diastolic blood pressure (Bokslag 2017, Drost 2012, Ernawati 2019), current smoking (Bijl 2022; FGR w/ 67% stillbirth, Bokslag 2017, Breetveld 2015; PE w/ 7% stillbirth, Drost 2012, Ernawati 2019, Ghossein-Doha 2016; PE w/ 8% stillbirth, Markovitz 2021; PTB w/ 10% stillbirth), BMI (Bijl 2022, Bokslag 2017, Breetveld 2015, Drost 2012, Ernawati 2019, Ghossein-Doha 2016), total cholesterol (Bijl 2022, Bokslag 2017, Breetveld 2015, Drost 2012, Ernawati 2019), HDL (Ernawati 2019), LDL (Ernawati 2019), triglycerides (Ernawati 2019), and fasting glucose (Ernawati 2019). While the evidence is certainly in support of elevated lifetime CVD risk in this patient population, it should be noted that stillbirth is currently not included as an indication for postpartum CVD risk assessment, however this should likely be revisited moving forward [21].

Placental Abruption

Only one study evaluated placental abruption independently. The placental abruption study demonstrated evidence of non-optimal systolic blood pressure (n=1; 100%), BMI (n=1; 100%), and total cholesterol (n=1; 100%). These findings are also in line with the morbidity-based systematic review of Smith et al. where an 82% increase in CVD events was observed [8]. Likewise, an additional 3 studies reported co-morbid placental abruption proportions ranging

from 4.7-11%. These studies also presented evidence of non-optimal systolic blood pressure (VanRijn 2013; PE w/ 11% placental abruption), smoking (Catov et al. PTB w/ a proportion of placental abruption, VanRijn 2013, VanRijn 2014; PE w/ 4.7% placental abruption), BMI (Catov et al. 2013, VanRijn 2013, VanRijn 2014), and total cholesterol (VanRijn 2013, VanRijn 2014). As with stillbirth, placental abruption is not currently recognized as an indication for postpartum screening referrals [21]. We would argue that more focused research in this area is warranted and based on the limited literature available re-visiting of clinical guidelines for CVD risk assessment following pregnancy may be required to include placental abruption.

Clinical Interpretation

This review demonstrates further evidence that PMDs are collectively associated with increased risk for CVD. As current guidelines only recommend postpartum CVD risk assessments for women with a history of preeclampsia, HELLP, gestational diabetes, fetal growth restriction, and/or pre-term birth, improvement to clinical guidelines for CVD risk screening, prediction, and prevention is highly warranted for women with a history of PMDs [21]. As demonstrated further in our results, stillbirth and placental abruption also follow similar postpartum CVD risk profiles to the other PMDs listed above and should therefore also be included in clinical recommendations for additional screening. We do note however that a small proportion of studies on preterm birth, stillbirth, and placental abruption were included in our review and should also be further explored to understand their independent association with CVD risk. In addition, as demonstrated in our results, many PMDs occur in one same gestation, allowing for the difficulties in characterizing the independent relationship between each PMD type and maternal risk for CVD. Further, as some studies have demonstrated the risk for CVD is increased

in cases of co-morbid PMDs [22]. Collectively, these results demonstrate the importance of taking a detailed obstetric history when assessing cardiovascular risk in women.

Strengths and Limitations

This review involved a comprehensive, robust search strategy of 4 large databases. This strategy was developed in conjunction with a content expert and medical librarian. This review also included 3 reviewers where every step was completed by at least 2 of the 3 and disagreements were settled by a discussion with all reviewers. Further, this review characterized the CVD risk profiles of 6 types of PMD. To our knowledge, this is the first time this has been presented. We also collected a wide variety of variables in order to capture both clinical and biochemical CVD risk markers. However, this review is not without limitations. Although we included all eligible studies from 1 month postpartum onwards, we observed considerable differences in risk assessment timing between PMD types. This limits some ability for comparisons between PMD type notably for the less commonly studied diseases (stillbirth, PTB, and placental abruption). This body of work is also correlation-based, limiting the potential for any causative conclusions. It is strongly encouraged that mechanistic studies using a variety of research models (such as in vitro cell/tissue culture and animal models) are carried out by the research community, allowing for a better understanding of these relationships and importantly for effective interventional and therapeutic targeting. Further, as demonstrated in our quality assessment, a proportion of studies did not adjust their analyses for potential confounding variables (such as age and ethnicity), and as such, the results presented in our review are unadjusted. In lieu of this, many studies did match cases and controls prior to study commencement. Another limitation is the high degree of co-morbidity between PMDs. This made

it difficult to determine the relationship between CVD risk and PMDs individually. Comorbid PMDs was also not reported in many studies (not indicating if preeclampsia cases were also preterm or involved stillbirth or fetal growth restriction/small for gestational age). On the other hand, some studies did report comorbid PMDs but did not evaluate their effect independently. An additional weakness was the disproportionally small number of studies on stillbirth and placental abruption. A smaller proportion of studies also went on to calculate a CVD risk score. Major differences in study design was also observed here where differing prediction models (5-year, 10-year, 20-year, 30-year, lifetime, and QRISK) and data presentation (odd ratios for high risk screening, raw risk scores, and percentage of sample screeded as high-risk) made for difficulties in drawing conclusions. In addition, the majority of studies included came from Western countries and included primarily Caucasian participants. Thus, our ability to generalize our results to all populations is limited, especially as ethnicity is known to influence CVD. Finally, we did not access publication bias or search grey literature.

Conclusions

Women with a history of PMDs - preeclampsia, gestational diabetes, fetal growth restriction/small for gestational age, preterm birth, stillbirth, and placental abruption - demonstrated evidence of increased markers of CVD risk at varying timepoints in the postpartum period. As such, a detailed obstetric history beyond the occurrence of hypertensive disorders of pregnancy is warranted. Further, the use of elevations in systolic blood pressure, BMI, current smoking status, and total cholesterol may be CVD risk markers specifically relevant to this population of young women. Improved risk assessment tools and triage strategies in the postpartum period should be explored for women with a history of PMDs.

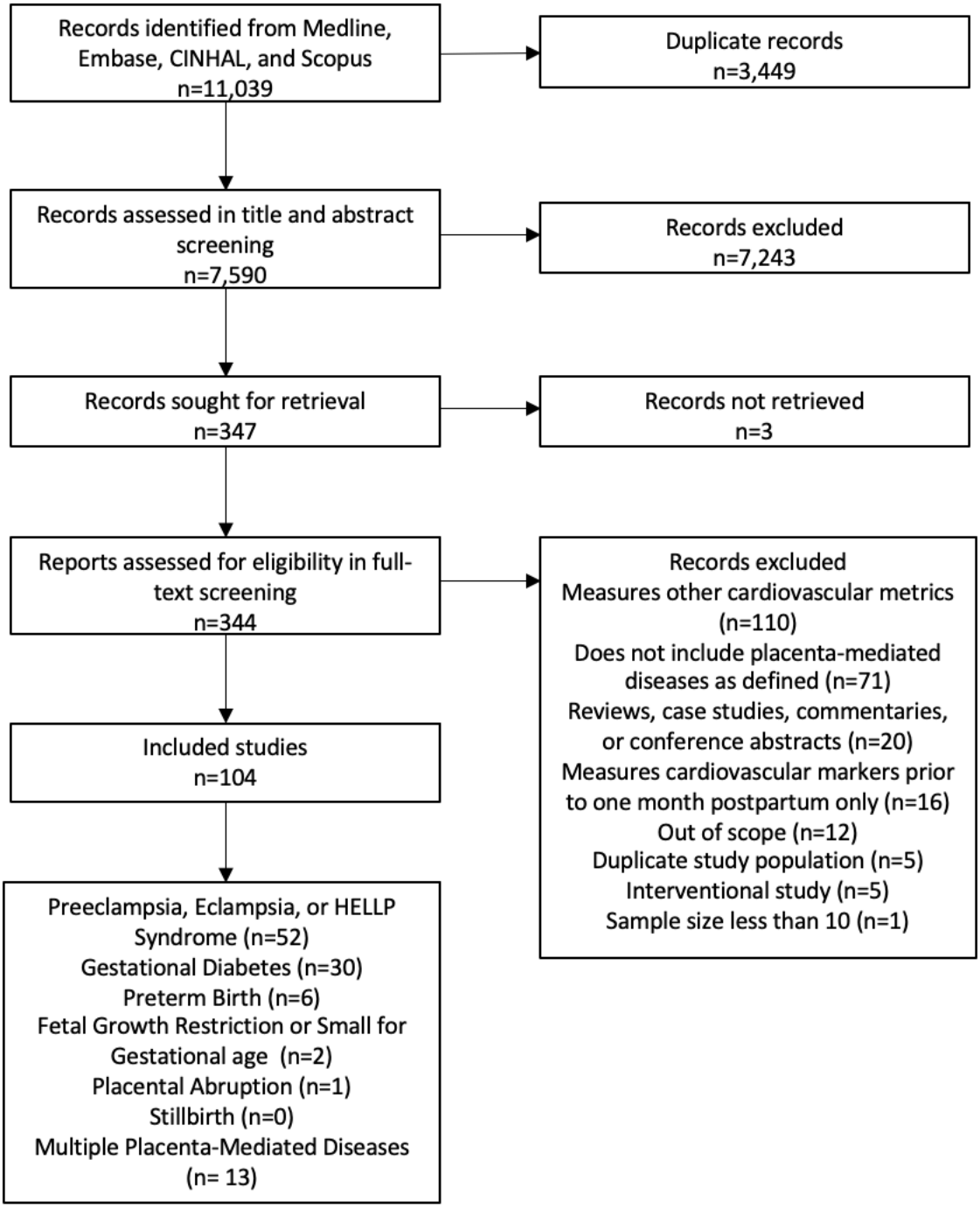


Figure 2.1 PRISMA Study Selection Diagram. 11,039 records were identified from Scopus, Medline, CINAHL, and Embase based on our search criteria. Of this, 347 met our inclusion criteria for full-text screening and 104 for data extraction.

Table 2.2 Summary Characteristics of Included Studies.

PMD Type	Number of Studies			PMD Sample Size Range (n)	Range of Risk assessment Timing (y)	Assessed Traditional Risk Measures[1] (n)	Calculated Risk Score[2] (n)
	Cohort (n)	Case-Control (n)	Cross-Sectional (n)				
Preeclampsia / HELLP	29	4	19	30-27300	0.14-35	52	15
Gestational Diabetes Mellitus	10	6	14	37-39581	0.12-20	30	3
Fetal Growth Restriction/ Small for gestational age	2	0	0	31-537	0.3-2.33	2	0
Preterm Birth	4	0	2	487-116429	8.5-25	6	1
Placental Abruption	1	0	0	154	0.67	1	0
Combination of PMDs	10	0	3	21-13861	0.23-25	13	5

[1]Includes measures of pre-pregnancy BMI, blood pressure, fasting glucose, HDL, LDL, triglycerides, or total cholesterol

[2]Includes 10-year, 20-year, 30-year, or lifetime CVD risk estimation, or risk for metabolic syndrome

Figure 2.2 Average Postpartum Systolic Blood Pressure by Type of Placenta-Mediated Disease. The mean (standard deviation) or median (interquartile range) for postpartum systolic blood pressure are presented for each study that included this measure. Timing of measurement is indicated by grey shading. A total of 103 measurements of systolic blood pressure were recorded. The range of blood pressure measurement timing was 0.14-35 years postpartum following preeclampsia; 0.12-20 years following gestational diabetes; 0.3-25 years following fetal growth restriction or small for gestational age; and 8.5-25 years following preterm birth. Systolic blood pressure above 120 mmHg was considered non-optimal in accordance with the lifetime CVD risk assessment threshold. 49 studies out of 103 were above this threshold (50.0%).

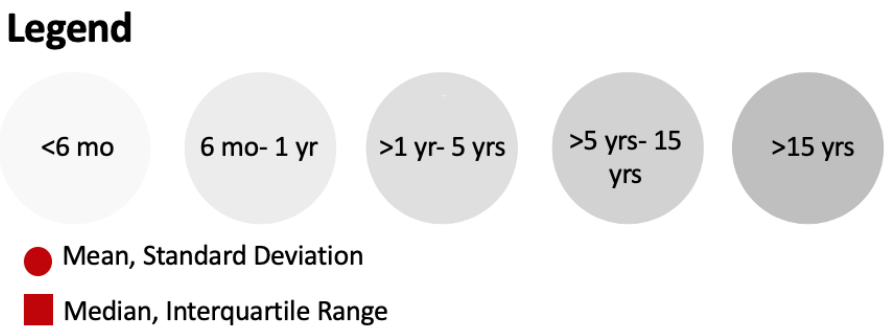


Figure 1. Postpartum Systolic Blood Pressure by Placenta-Mediated Disease Type

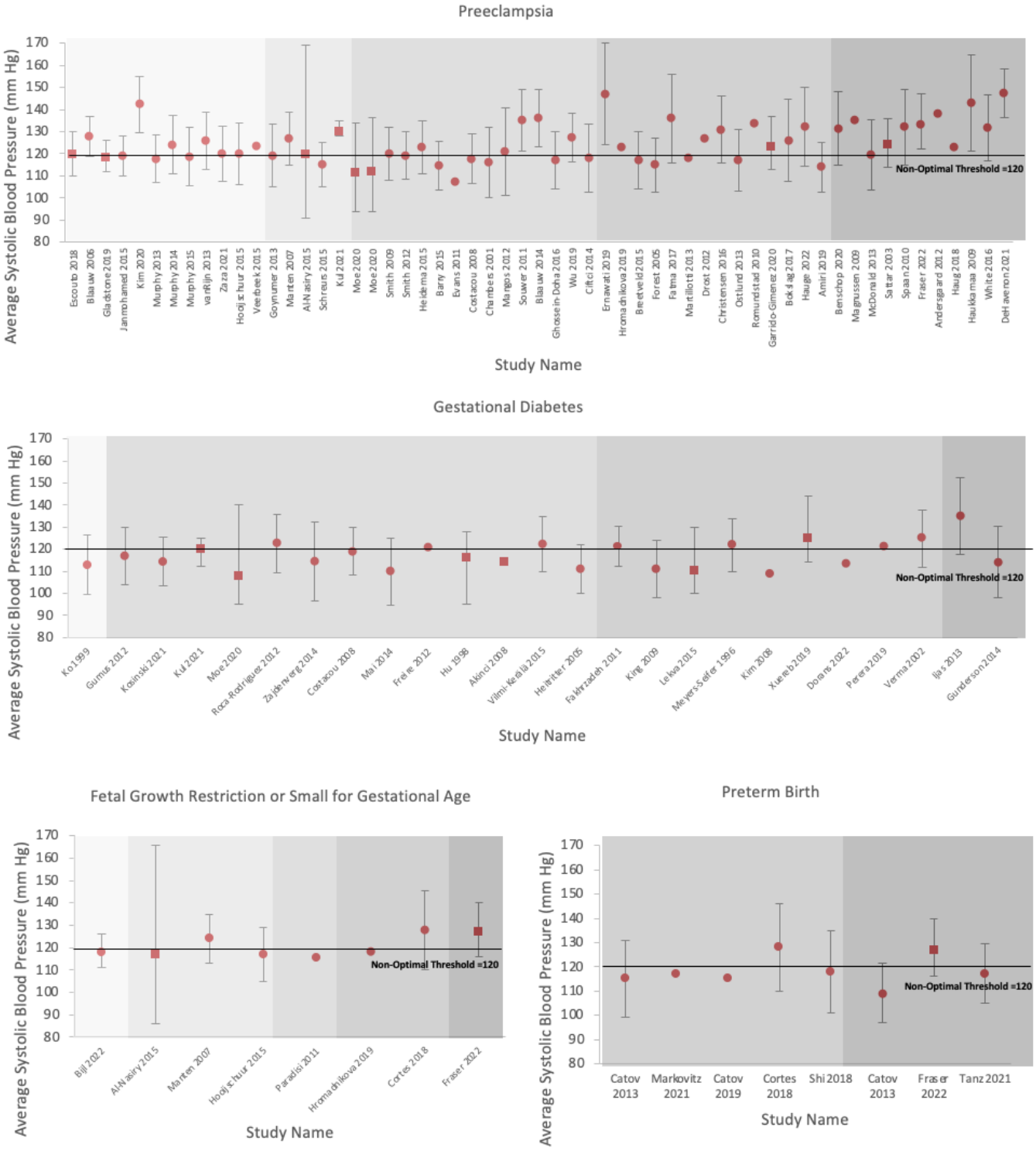


Figure 2.3 Average Postpartum Diastolic Blood Pressure by Type of Placenta-Mediated Disease. The mean (standard deviation) or median (interquartile range) for postpartum diastolic blood pressure are presented for each study that included this measure. Timing of measurement is indicated by grey shading. A total of 99 measurements of systolic blood pressure were recorded. The range of blood pressure measurement timing was 0.14-35 years postpartum following preeclampsia; 0.12-20 years following gestational diabetes; 0.3-14.3 years following fetal growth restriction or small for gestational age; and 8.5-25 years following preterm birth. Diastolic blood pressure above 80 mmHg was considered non-optimal in accordance with the lifetime CVD risk assessment threshold. 22 studies out of 99 were above this threshold (22.0%).

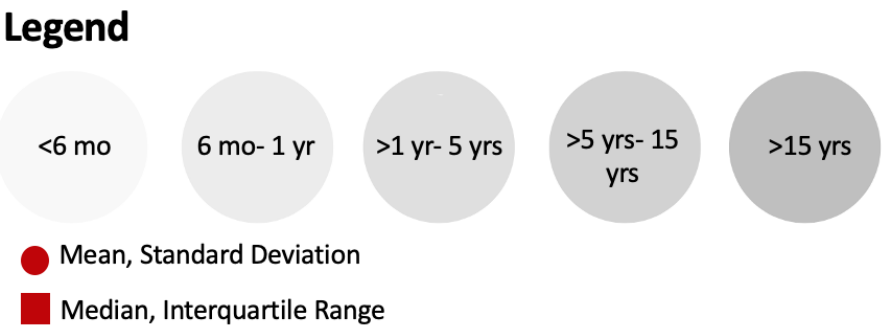


Figure 2. Postpartum Diastolic Blood Pressure by Placenta-Mediated Disease Type

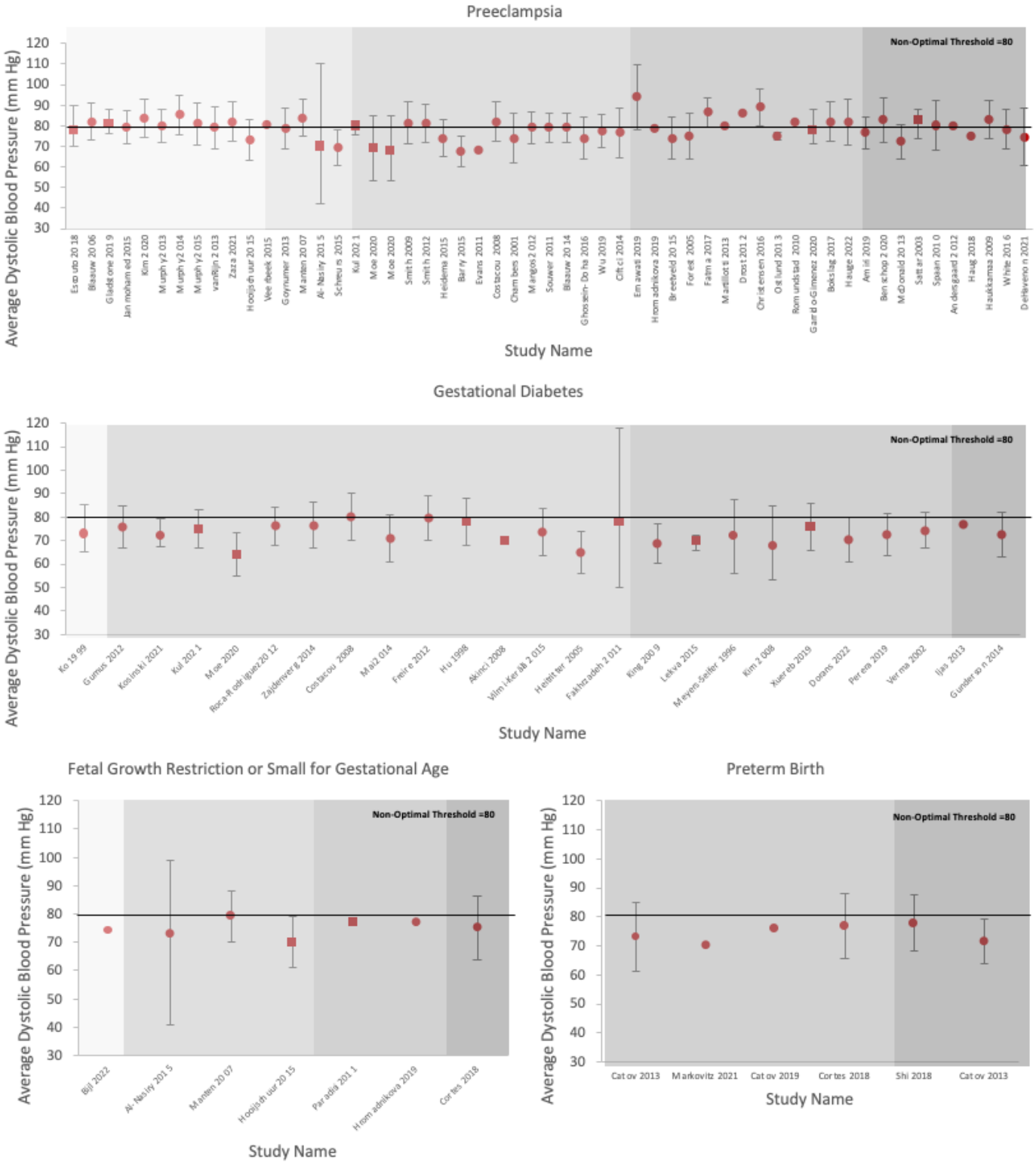


Figure 2.4 Average Postpartum* BMI by Type of Placenta-Mediated Disease. The mean (standard deviation) or median (interquartile range) for postpartum BMI are presented for each study that included this measure. Timing of measurement is indicated by grey shading. A total of 108 measurements of BMI were recorded. The range of BMI measurement timing was 0.14-35 years postpartum following preeclampsia; 0.12-20 years following gestational diabetes; 0.3-14 years following fetal growth restriction or small for gestational age; and 8.5-20 years following preterm birth. Average BMI above 25 kg/m^2 were considered overweight in accordance with the 10-year CVD risk assessment threshold. 86 studies out of 108 were above this threshold (79.6%). *8 studies did not include postpartum BMI measurements and pre-pregnancy BMI is included instead. These studies are denoted with a star after the study name.

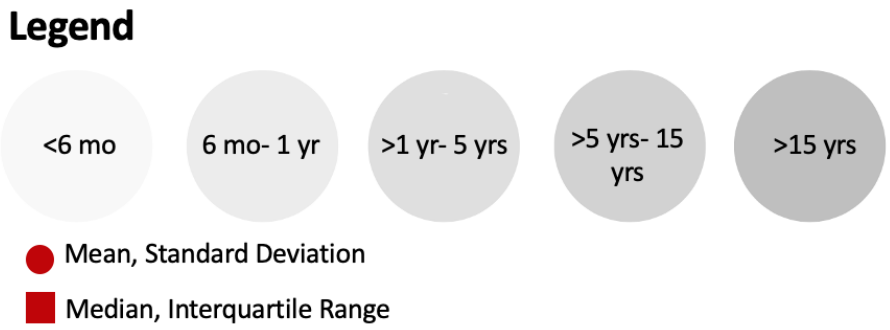


Figure 3. Body Mass Index by Placenta-Mediated Disease Type

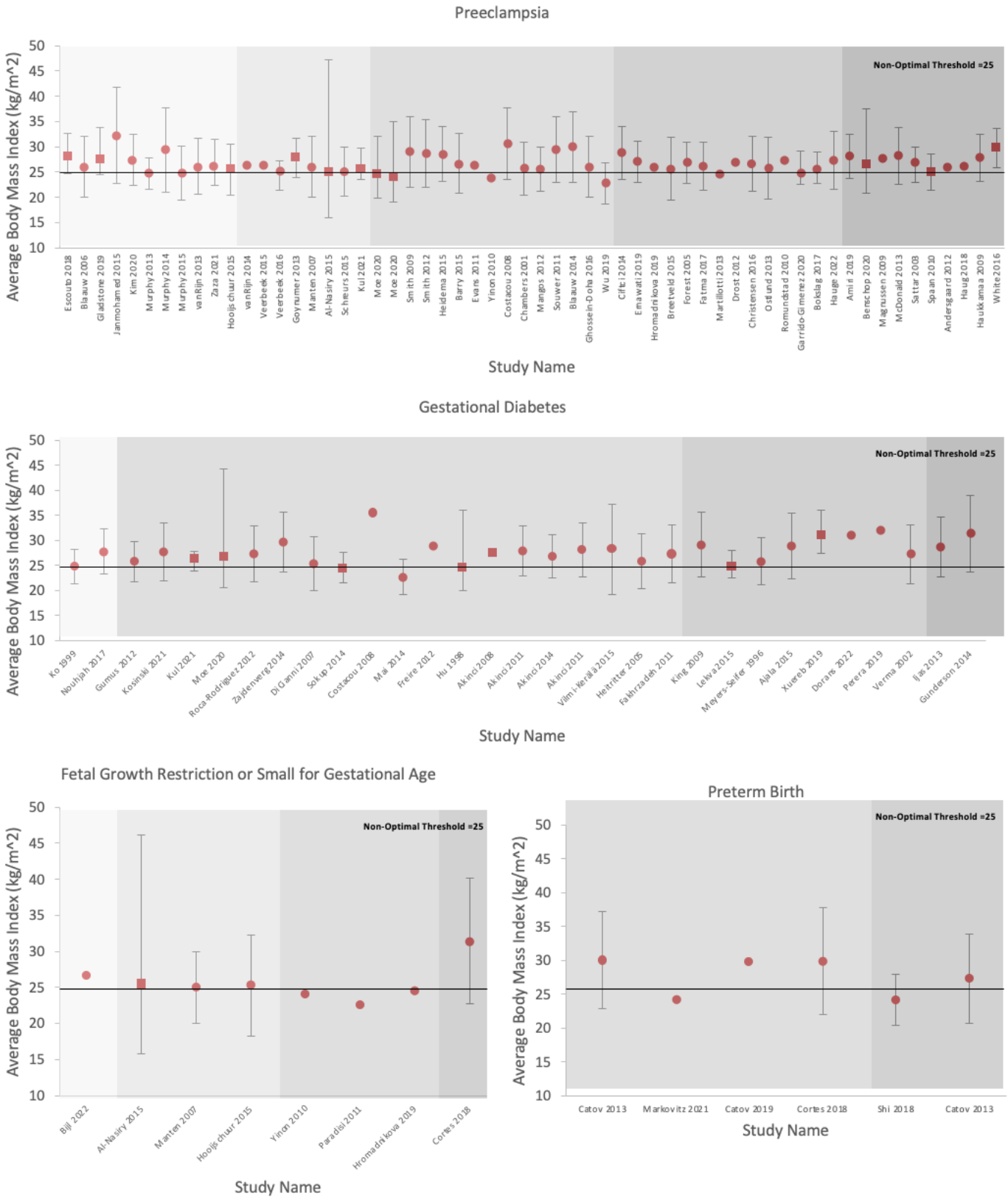


Figure 2.5 Average Postpartum Waist Circumference by Type of Placenta-Mediated Disease. The mean (standard deviation) or median (interquartile range) for postpartum waist circumference are presented for each study that included this measure. Timing of measurement is indicated by grey shading. A total of 48 measurements of waist circumference were recorded. The range of BMI measurement timing was 0.5-25.4 years postpartum following preeclampsia; 1-20 years following gestational diabetes; and 8.5-20 years following preterm birth. Average waist circumference above 88 cm was considered non-optimal in accordance with the 10-year CVD risk assessment threshold. 30 studies out of 48 were above this threshold (62.5%).

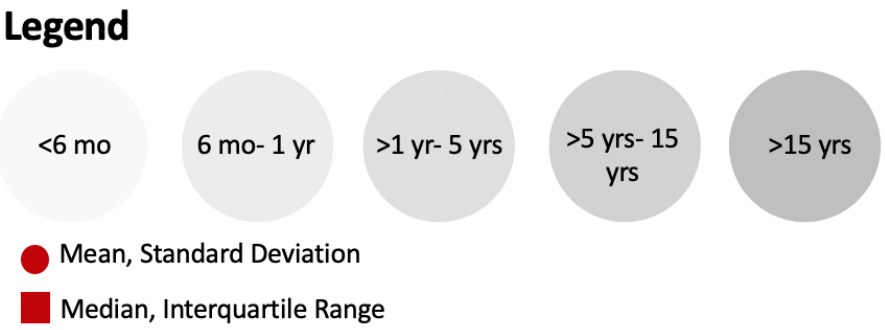


Figure 4. Waist Circumference by Placenta-Mediated Disease Type

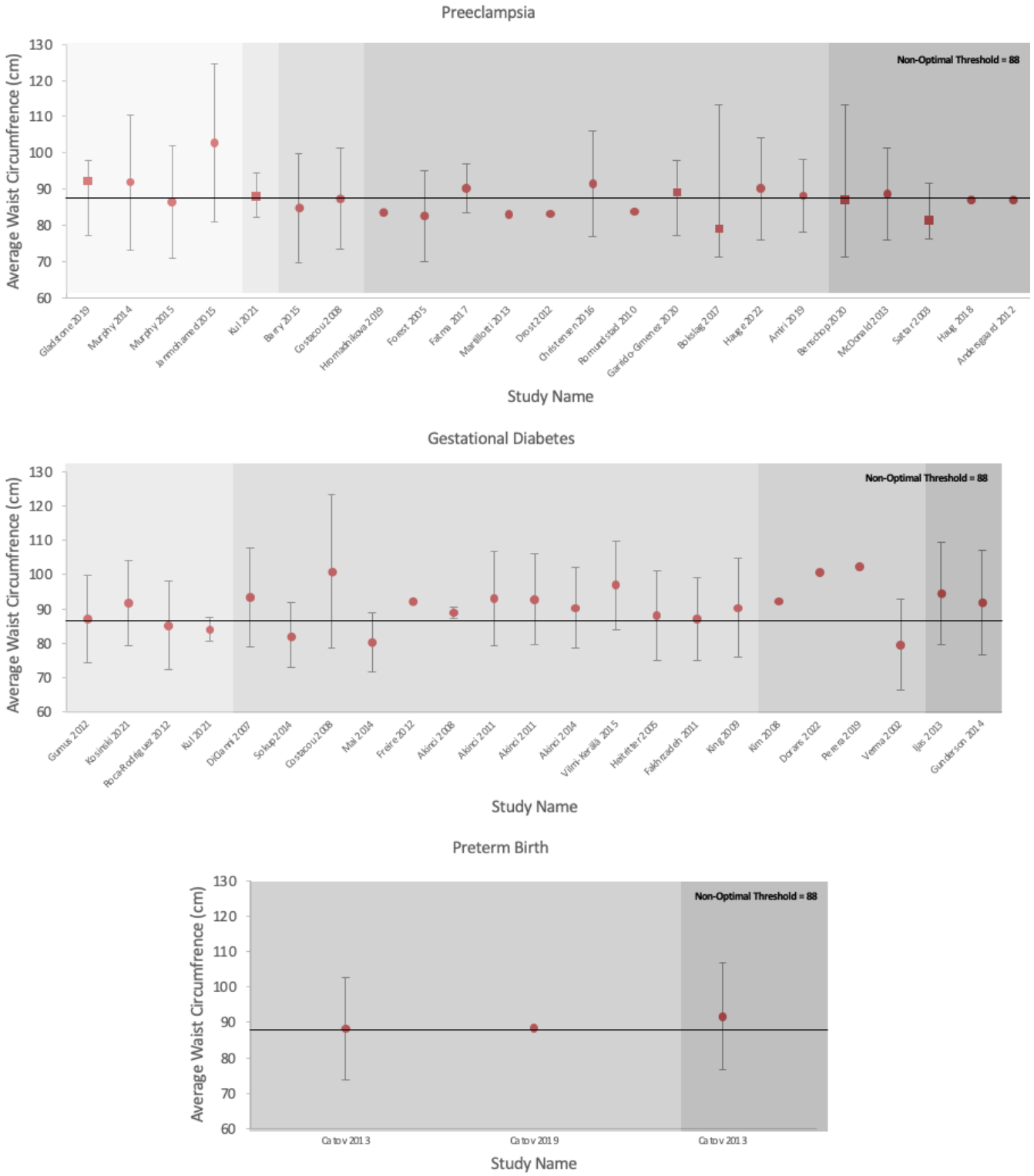


Figure 2.6 Average Postpartum Cholesterol Levels by Type of Placenta-Mediated Disease. The mean (standard deviation) or median (interquartile range) for postpartum waist circumference are presented for each study that included this measure. Timing of measurement is indicated by grey shading. A total of 98 measurements of cholesterol were recorded. The range of cholesterol measurement timing was 0.14-35 years postpartum following preeclampsia; 0.12-20 years following gestational diabetes; 0.2-25 years following fetal growth restriction or small for gestational age; and 9-25 years following preterm birth. Cholesterol levels above 4.65 mmol/L were considered non-optimal in accordance with the lifetime CVD risk assessment threshold. 79 studies out of 98 were above this threshold (80.6%).

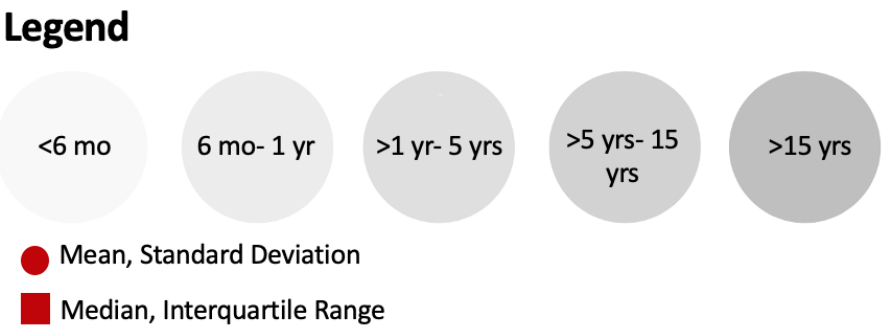


Figure 5. Total Cholesterol by Placenta-Mediated Disease Type

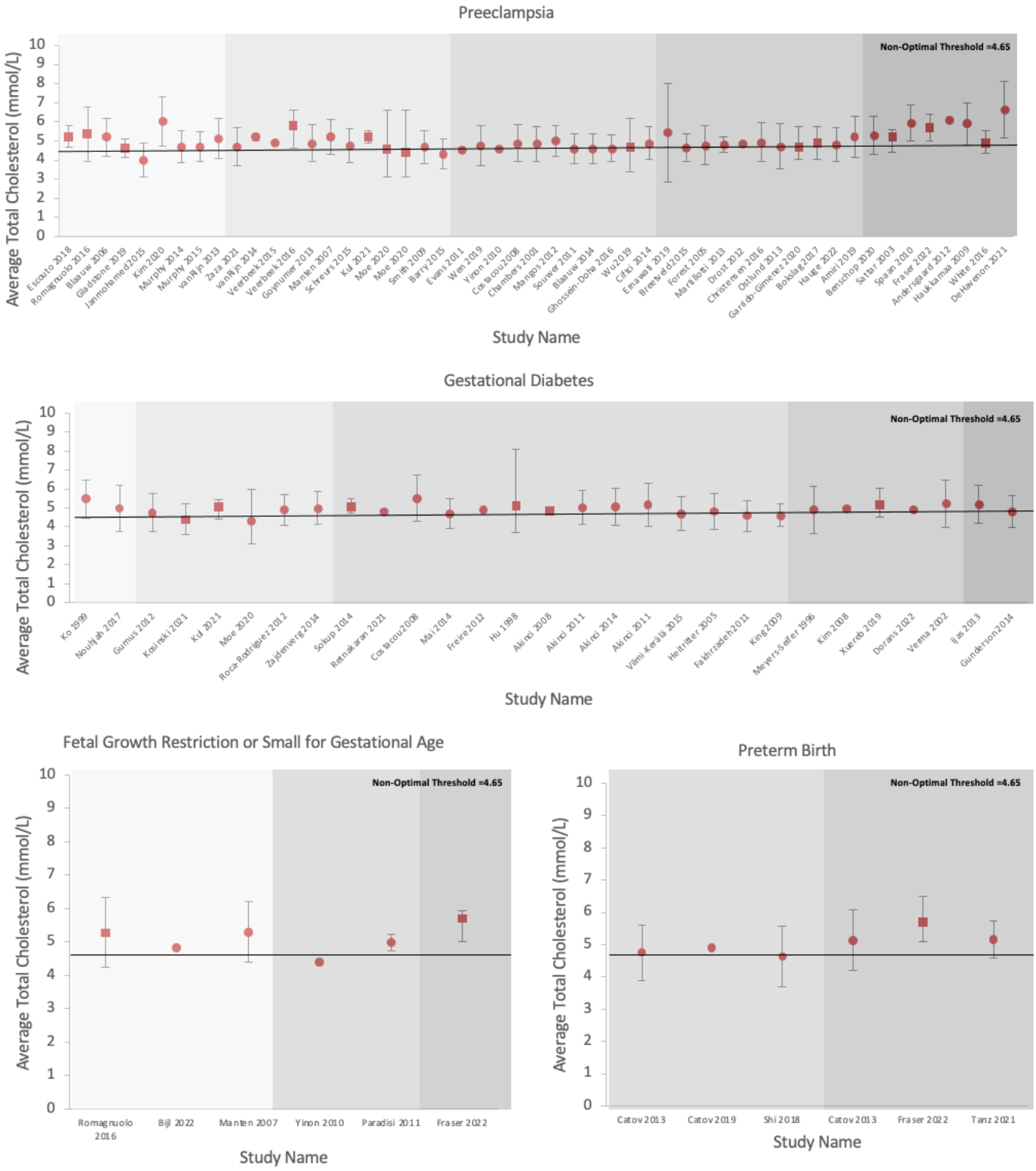


Figure 2.7 Average Postpartum HDL Levels by Type of Placenta-Mediated Disease. The mean (standard deviation) or median (interquartile range) for postpartum waist circumference are presented for each study that included this measure. Timing of measurement is indicated by grey shading. A total of 108 measurements of HDL were recorded. The range of HDL measurement timing was 0.14-35 years postpartum following preeclampsia; 0.12-20 years following gestational diabetes; 0.2-14 years following fetal growth restriction or small for gestational age; and 9-25 years following preterm birth. HDL levels below 1.3 mmol/L were considered non-optimal in accordance with the lifetime CVD risk assessment threshold. 26 studies out of 108 were below this threshold (24.1%).

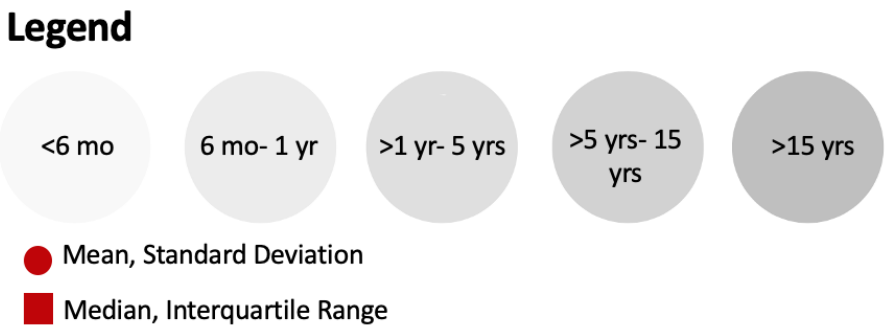


Figure 6. HDL by Placenta-Mediated Disease Type

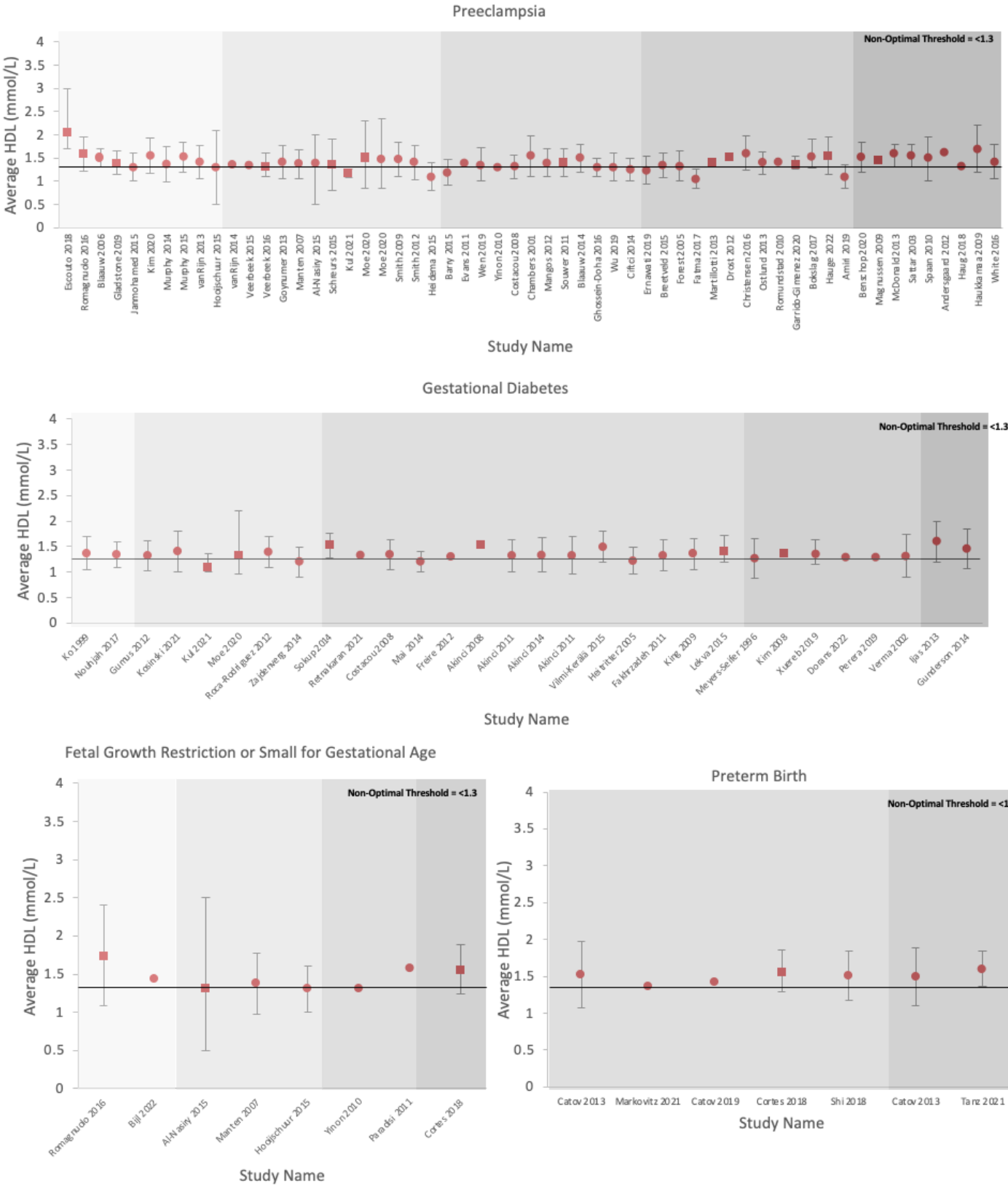


Figure 2.8 Average Postpartum LDL Levels by Type of Placenta-Mediated Disease. The mean (standard deviation) or median (interquartile range) for postpartum waist circumference are presented for each study that included this measure. Timing of measurement is indicated by grey shading. A total of 94 measurements of LDL were recorded. The range of LDL measurement timing was 0.14-35 years postpartum following preeclampsia; 0.12-20 years following gestational diabetes; 0.2-25 years following fetal growth restriction or small for gestational age; and 9-25 years following preterm birth. LDL levels above 3.5 mmol/L were considered non-optimal in accordance with the 10-year CVD risk assessment threshold. 5 studies out of 94 were above this threshold (5.4%)

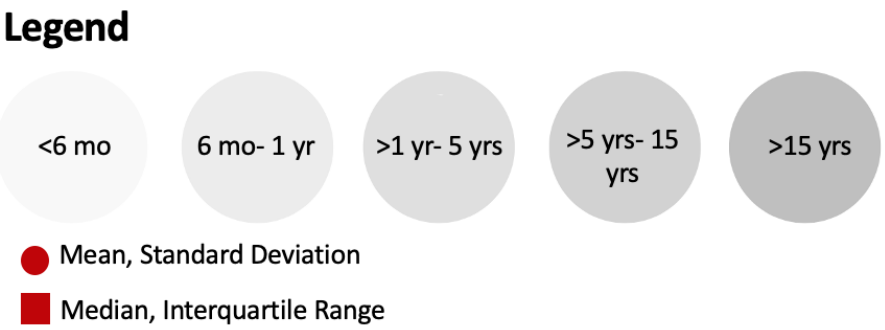


Figure 7. LDL by Placenta-Mediated Disease Type

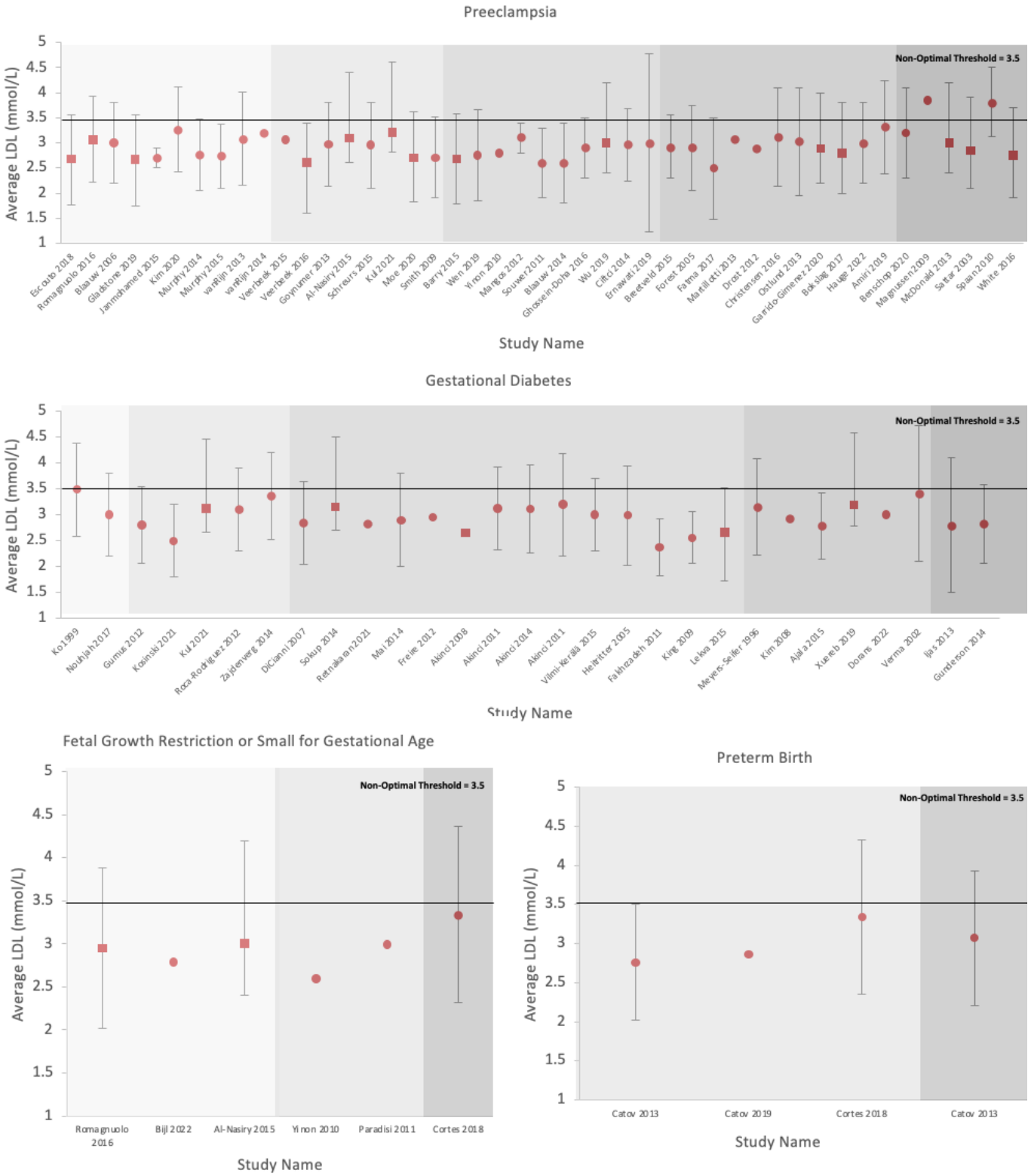


Figure 2.9 Average Postpartum Triglyceride Levels by Type of Placenta-Mediated Disease. The mean (standard deviation) or median (interquartile range) for postpartum waist circumference are presented for each study that included this measure. Timing of measurement is indicated by grey shading. A total of 103 measurements of triglycerides were recorded. The range of triglyceride measurement timing was 0.14-35 years postpartum following preeclampsia; 0.12-20 years following gestational diabetes; 0.2-14 years following fetal growth restriction or small for gestational age; and 9-25 years following preterm birth. Triglyceride levels above 1.7 mmol/L were considered non-optimal in accordance with the lifetime CVD risk assessment threshold. 7 studies out of 103 were above this threshold (6.8%).

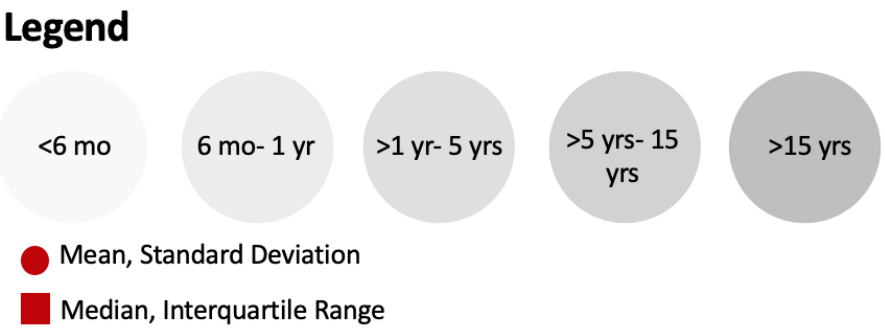


Figure 8. Triglycerides by Placenta-Mediated Disease Type

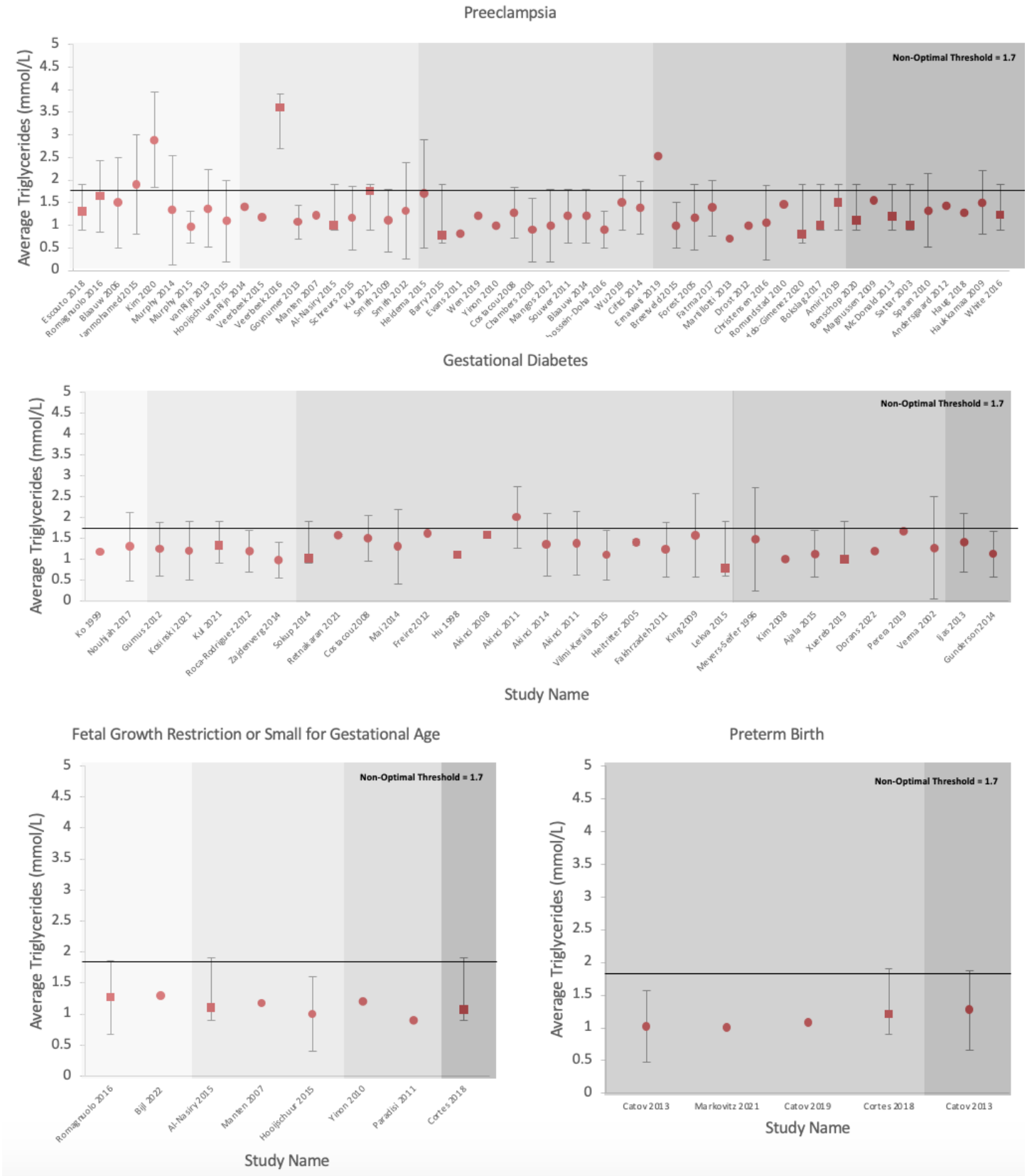


Figure 2.10 Average Postpartum Glucose Levels by Type of Placenta-Mediated Disease. The mean (standard deviation) or median (interquartile range) for postpartum waist circumference are presented for each study that included this measure. Timing of measurement is indicated by grey shading. A total of 81 measurements of glucose levels were recorded. The range of glucose measurement timing was 0.14-35 years postpartum following preeclampsia; 0.12-20 years following gestational diabetes; 0.2-14 years following fetal growth restriction or small for gestational age; and 20 years following preterm birth. Glucose levels above 5.6 mmol/L were considered non-optimal in accordance with the lifetime CVD risk assessment threshold. 15 studies out of 81 were above this threshold (18.5%).

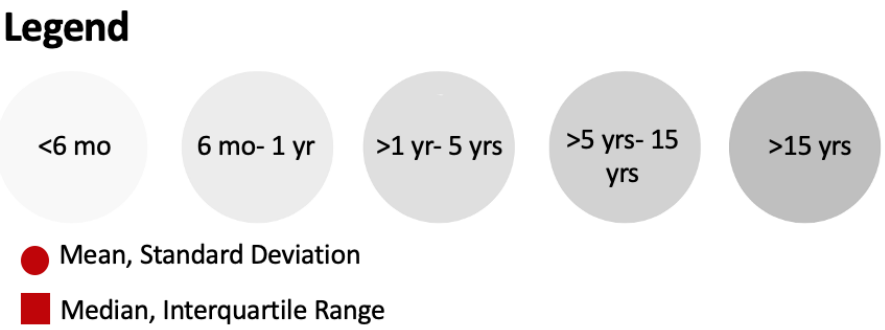


Figure 9. Fasting Glucose by Placenta-Mediated Disease Type

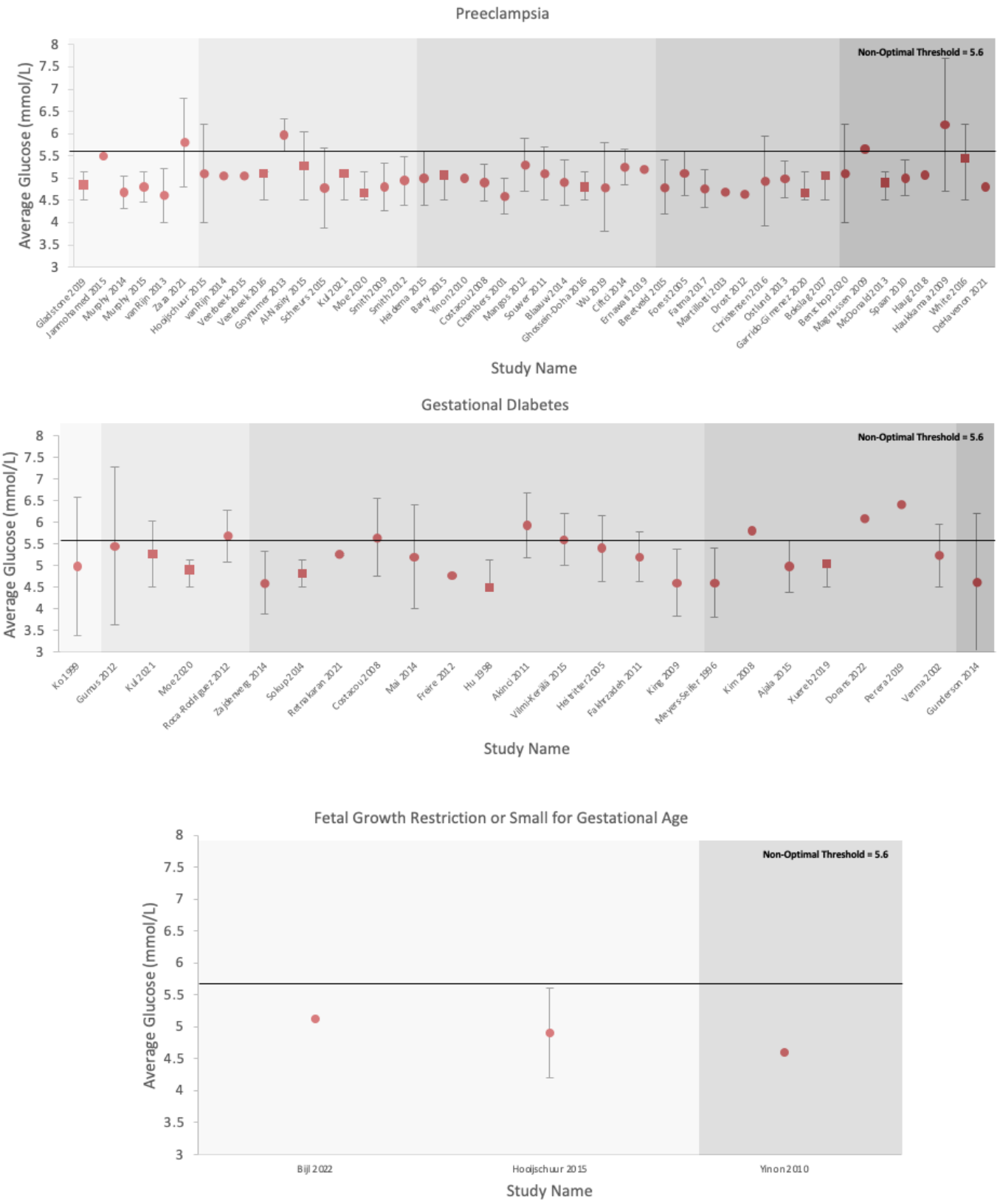
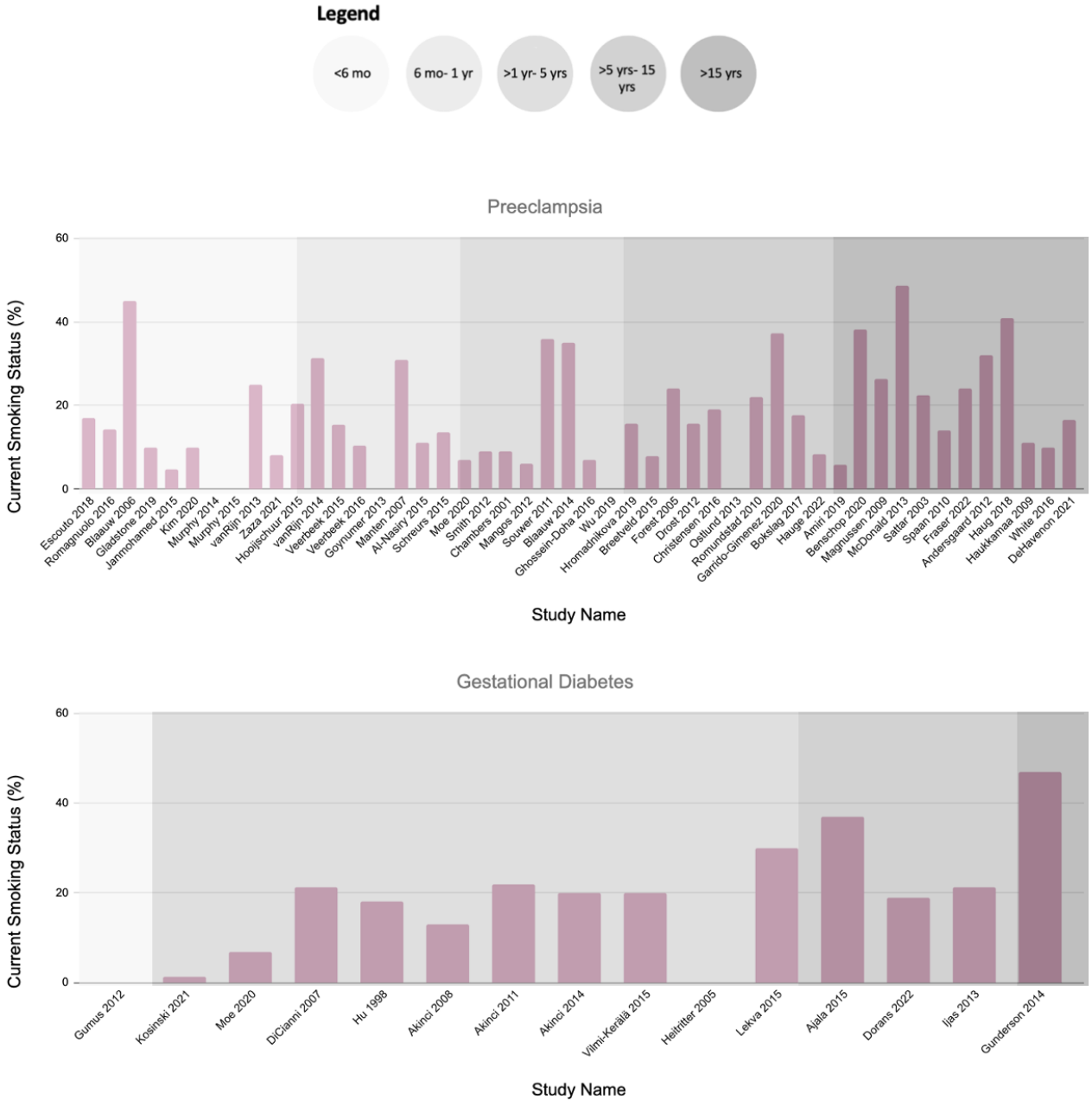
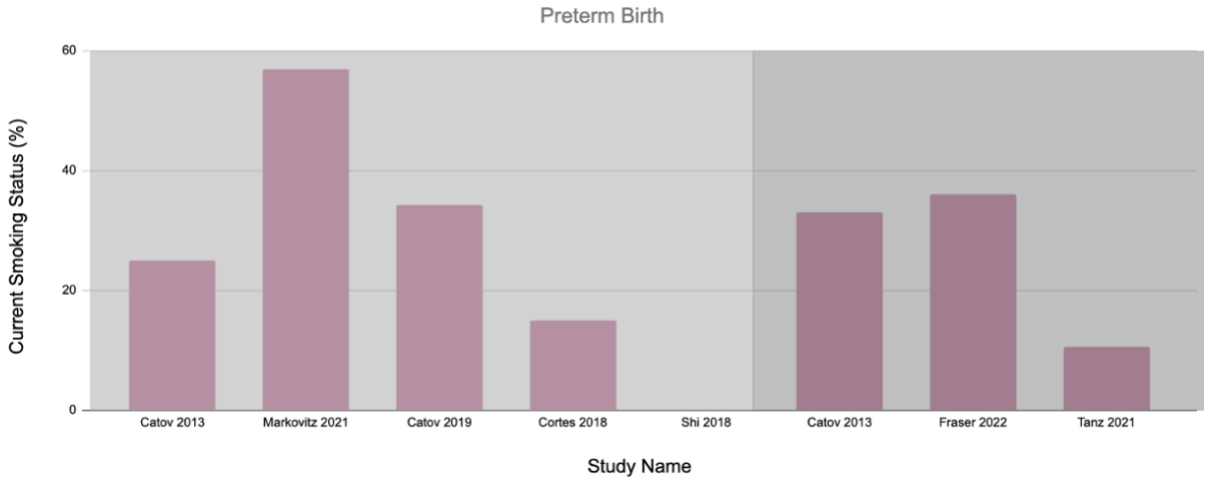


Figure 2.11 Average Postpartum Smoking by Type of Placenta-Mediated Disease. The percentage of current smokers was recorded for each study. Timing of measurement is indicated by grey shading. A total of 85 measurements of current smoking were recorded. The range of smoking measurement timing was 0.14-35 years postpartum following preeclampsia; 0.12-20 years following gestational diabetes; 0.23-25 following fetal growth restriction or small for gestational age; and 9-25 years following preterm birth. Current smoking is considered a major risk for CVD according to the lifetime CVD risk assessment. 10 studies excluded any smoking participants and are thus recorded as 0%.





Supplementary Material

S1. Quality Assessment

Study ID	1 (max = ★)	2 (max = ★★	3 (max = ★)	4 (max = ★★)	5 (max = ★)	6 (max = ★★)	7 (max = ★)	8 (max = ★★)	9 (max = ★★)	10 (max = ★★)	11 (max = ★)	12 (max = ★)	Total Score (/18)
Gestational Diabetes													
Ajala 2015	★	★	★	-	-	★	★	-	★★	★★	★	★	11
Akinci 2008	★	★	★	★★	★	★	-	-	★★	★★	★	★	13
Akinci 2011	★	★	★	★★	★	★	★	-	★★	★★	★	★	14
Akinci 2011	★	★	★	★★	★	★★	★	★★	★★	★★	★	★	17
Akinci 2014	★	★	★	★★	★	★	★	-	★★	★★	★	★	14
DiCianni 2007	★	★	★	★★	★	-	-	-	★★	★★	★	★	12
Dorans 2022	-	★★	-	★	★	★★	★	-	★★	★★	★	★	13

Fakhrzadeh 2011	★	★	★	★★	★	★	-	★★	★★	★★	★	★	15
Freire 2012	★	★	★	★★	★	★★	-	-	★★	★★	★	★	14
Gumus 2012	★	★★	★	★★	★	★	-	-	★★	★★	★	★	14
Gunderson 2014	-	★★	★	★	★	-	★	★	★★	★★	★	★	13
Heitritter 2005	★	★	★	★★	★	-	★	-	★★	★★	★	★	13
Hu 1998	★	★	-	★★	★	★	-	-	-	★★	★	★	10
Ijas 2013	★	★	-	★★	★	-	-	-	★★	★★	★	★	11
Kim 2008	-	★	★	★	★	★★	-	-	★★	-	★	★	10
King 2009	-	★★	★	★★	★	★★	-	-	★★	★★	★	★	14
Ko 1999	★	★★	-	★★	★	★★	★	-	-	★★	★	★	13
Kosinski 2021	★	★	★	-	-	-	★	-	★★	★★	-	★	9
Lekva 2015	★	★	★	-	-	-	-	-	★★	★★	★	★	9
Mai 2014	★	★★	★	★★	★	-	-	-	★★	★★	★	★	13
Meyers-Seifer 1996	★	★		★★	★	-	-	-	★★	★★	★	★	11
Nouhjah 2017	★	★★	-	★★	★	★★	★	★	★	★★	★	★	15
Perera 2019	-	★★	-	★	★	★★	★	-	★★	★★	★	★	13
Retnakaran 2021	★	★★	-	★★	★	★★	★	-	★★	★★	★	★	15
Roca-Rodriguez 2012	★	★	★	★★	★	★	-	-	★★	★★	★	★	13
Sokup 2014	★	★	-	-	-	-	★	-	★	★★	-	★	7
Verma 2002	★	★	-	★	★	★	★	-	★★	★★	★	★	12
Vilmi-Kerälä 2015	★	★	★	★★	★	★	★	-	★★	★★	★	★	14
Xuereb 2019	★	★	-	★★	★	-	-	★	★★	★★	★	★	12
Zajdenverg 2014	★	★	★	★★	★	-	-	★★	★★	★★	★	★	14
Placental Abruption													
Veerbeek 2013	★	★	★	★★	★	-	★	-	★	★★	★	★	12
Preeclampsia													
Amiri 2019	-	★★	★	★	★	-	★	★★	-	★★	★	★	12
Andersgaard 2012	-	★★	-	★	★	★★	★	★★	★★	★★	★	★	15
Barry 2015	★	★	★	★★	★	★	★	-	★★	★★	★	★	14
Benschop 2020	★	★	★	★★	★	★	★	★★	★★	★★	★	★	16
Blaauw 2014	★	★	-	★★	★	-	-	★	★	★★	★	★	11
Bokslag 2017	★	★	★	★★	★	★	★	★★	★★	★★	★	★	16
Breetveld 2015	★	★	★	★★	★	-	★	-	★★	★★	★	★	13
Chambers 2001	★	★★	★	★★	★	-	★	★★	★	★★	★	★	15
Christensen 2016	★	★	-	★★	★	★	-	-	★★	★★	★	★	12
Ciftci 2014	★	★	★	★★	★	-	-	★	★★	★★	★	★	13
DeHavenon 2021	-	★	-	★	★	-	★	★	★★	★★	★	★	11
Drost 2012	★	★	-	★★	★	★	★	-	★★	★★	★	★	13

Ernawati 2019	★	★	★	★★	★	-	-	★	★★	★★	★	★	13
Escouto 2018	★	★★	★	★★	★	-	-	-	-	★★	★	★	11
Evans 2011	★	★	★	★★	★	-	-	★★	★★	★★	★	★	14
Fatma 2017	★	★★	★	★★	★	★	-	-	★★	★★	★	★	14
Forest 2005	★	★	★	★★	★	★	-	-	★★	★★	★	★	13
Garrido-Gimenez 2020	★	★	★	★★	★	-	-	-	★★	★★	★	★	12
Ghossein-Doha 2016	★	★	★	★★	★	-	★	★	★★	★★	★	★	14
Goynumer 2013	★	★	★	★★	★	-	-	-	★★	★★	★	★	12
Haug 2018	★	★★	-	★★	★	-	★	★	★★	★★	★	★	14
Haukkamaa 2009	★	★★	★	★★	★	-	★	★★	★★	★★	★	★	16
Heidema 2015	★	-	★	★★	★	★	-	-	★★	★★	★	★	12
Kim 2020	★	★	★	★★	★		-	★★	★	★★	★	★	13
Magnussen 2009	★	★★	★	★★	★	★★	★	★	★★	★★	★	★	17
Mangos 2012	★	★★	★	-	★	-	-	-	★★	★	★	★	10
Martillotti 2013	★	★	★	★★	★	★	-	-	★★	★★	★	★	13
McDonald 2013	★	★	★	★★	★	★	★	★	★★	★★	★	★	15
Moe 2020	★	★	★	★★	-	-	★	-	★★	★★	★	★	12
Murphy 2013	★	★	★	★★	★	-	-	-	★	★★	★	★	11
Murphy 2014	★	★	★	★★	★	-	-	★	★	★★	★	★	12
Murphy 2015	★	★	★	★★	★	-	-	-	★	★★	★	★	11
Ostlund 2013	★	★	★	★★	★	★	-	★	★★	★★	★	★	14
Romundstad 2010	★	★★	-	★★	★	★★	★	★	★★	★★	★	★	16
Sattar 2003	★	★	★	★★	★	★	-	-	★★	★★	★	★	13
Schreurs 2015	★	★	★	-	-	-	★	-	★	★★	-	★	8
Smith 2009	★	★	★	★★	★	★	★	★	★★	★★	★	★	15
Smith 2012	★	★	★	★★	★	-	★	★	★★	★★	★	★	14
Souwer 2011	★	★★	-	★★	★	-	-	-	★★	★★	★	★	12
Spaan 2010	★	★	★	★★	★	★	-	-	★★	★★	★	★	13
vanRijn 2013	★	★	★	★★	-	★	-	-	★	★★	★	★	11
vanRijn 2014	★	★	★	-	-	-	★	★★	★	★★	-	★	10
Veerbeek 2015	★	★	★	-		-	★	-	★★	★	-	★	8
Veerbeek 2016	★	★	-	★★	★	-	-	★	-	★★	★	★	10
Wen 2019	★	★★	★	★★	★	-	★	★★	★★	★★	★	★	16
White 2016	★	★	★	★★	★	★	-	★	★★	★★	★	★	14
Wu 2019	★	★	★	-	-	-	-	-	★★	★★	★	★	9
Zaza 2021	★	★	★	-	-	-	★	★	★	★★	-	★	9
Fetal Growth Restriction or Small for Gestational Age													
Bijl 2022	★	★	-	★★	★	-	★	★	-	★★	★	★	11

Paradisi 2011	★	★	★	★★	★	-	-	-	★★	★★	★	★	12
Preterm Birth													
Catov 2013	★	★	★	★★	★	-	★	-	★★	★★	★	★	13
Catov 2013	-	★	★	★	★	-	★	★	★★	★★	★	★	12
Catov 2019	★	★★	-	★★	★	-	★	★★	★★	★★	★	★	15
Markovitz 2021	★	★★	-	★★	★	-	★	★	★★	★★	★	★	14
Shi 2018	★	★★	★	★★	★	-	★	★	★★	★★	★	★	15
Tanz 2021	-	-	★	★	★	-	★	★	★★	★★	★	★	11
Multiple PMDs													
Al-Nasiry 2015	★	-	-	-	-	-	★	-	★	★★	-	★	6
Bhasin 2014	★	-	★	★★	★	★★	★	-	★★	★★	★	★	14
Blaauw 2006	★	★	★	★★	★	★	-	-	★	★★	★	★	12
Cortes 2018	-	★★	-	★	★	★★	★	-	★★	★★	★	★	13
Costacou 2008	★	★	★	★★	★	★	-	★	★★	★★	★	★	14
Fraser 2022	-	★	★	★★	★	★	-	★	★	★★	★	★	12
Kul 2021	★	★	★	★★	★	★★	-	-	-	★★	★	★	12
Gladstone 2019	★	★★	★	★★	★	★★	-	★	★	★★	★	★	15
Hauge 2022	★	★★	-	★★	★	★	★	-	★★	★★	-	★	13
Hooijschuur 2015	★	★	-	-	-	-	★	★	★	★★	-	★	8
Hromadnikova 2019	★	★★	-	★★	★	★★	★	★	★★	★★	★	★	16
Janmohamed 2015	★	★	-	-	-	-	-	-	★	★★	-	★	6
Moe 2020	★	★	★	★★	-	-	-	-	★★	★★	★	★	11
Mahendru 2013	★	★	★	★★	-	-	-	-	★★	★★	★	★	11
Manten 2007	★	★	-	★★	★	-	★	-	★	★★	★	★	11
Romagnuolo 2016	★	★	★	★★	★	-	★	★	★	★★	★	★	13
Yinon 2010	★	★	-	★★	★	-	-	-	★★	★★	★	★	11

Item 1 = Ascertainment of exposure (PMD); Item 2 = Representativeness of the participants; Item 3 = Demonstration that outcome of interest was not present at start of study; Item 4 = Definition of controls; Item 5 = Selection of controls; Item 6 = Comparability of cases and controls on the basis of the design or analysis; Item 7 = Sample size; Item 8 = Adequacy of follow up of participants; Item 9 = Was follow-up long enough for outcomes to occur?; Item 10 = Ascertainment of outcome; Item 11 = Same method of ascertainment for cases and controls; Item 12 = Statistical Tests used

S2. Extracted Study Characteristics from Included Studies

Study ID	Journal Name	Country	Study Design	Types of PMD Included	Risk Assessment Timing (yr)	Total Sample Size	Ethnicity	Maternal Age	Age At Follow-Up	Gestational Age at Delivery (wk)	Birth Weight (g) or Birth Weight Percentile
Ajala 2015	Diabetes Research and Clinical Practice	Canada	Cross sectional study	Gestational Diabetes	7.1	149	81% Caucasian	Unknown	39.2	Unknown	Unknown
Akinci 2008	Diabetes Research and Clinical Practice	Turkey	Cross sectional study	Gestational Diabetes	3.05	76	100% Caucasian	Unknown	34.72	Unknown	Unknown
Akinci 2011	Diabetes Research and Clinical Practice	Turkey	Cross sectional study	Gestational Diabetes	3.23	195	100% Caucasian	Unknown	35.21	Unknown	Unknown
Akinci 2011	Gynecological Endocrinology	Turkey	Cross sectional study	Gestational Diabetes	3.38	266	100% Caucasian	Unknown	31.87	Unknown	Unknown
Akinci 2014	Archives of Gynecology and Obstetrics	Turkey	Cross sectional study	Gestational Diabetes	3.36	190	100% Caucasian	Unknown	35.18	Unknown	Unknown
Al-Nasiry 2015	British Journal of Obstetrics and Gynaecology	The Netherlands	Cohort study	Pre-eclampsia; Small for Gestational Age	0.67 SGA; 0.92 PE	889	Unknown	31 SGA; 32 PE	Unknown	33 SGA; 34 PE	1000 SGA; 2083 PE
Amiri 2019	Pregnancy Hypertension	Iran	Cohort study	Pre-eclampsia	15	3022	Unknown	33.75	Unknown	Unknown	Unknown
Andersgaard 2012	American Journal of Obstetrics and Gynecology	Norway	Cross sectional study	Pre-eclampsia	25.4	9974	Unknown	Unknown	48.8	Unknown	Unknown
Barry 2015	American Journal of Obstetrics and Gynecology	United States of America	Cohort study	Pre-eclampsia	1.33	71	71.4% Caucasian, 16.3% Other, 12.2% Unknown	Unknown	33.4	Unknown	Unknown
Benschop 2020	Circulation	The Netherlands	Cross sectional study	Pre-eclampsia	16.3	902	100% Caucasian	Unknown	46	Unknown	Unknown
Bhasin 2014	Journal of Urban Health	India	Cross sectional study	Gestational Diabetes; Preterm birth	5	631	100% Punjabi	Unknown	32.76	Unknown	Unknown
Bijl 2022	Journal of Women's Health	The Netherlands	Cohort study	Fetal Growth Restiction	0.3	537	Unknown	Unknown	30.6	29	590.6; 2.7 percentile
Blaauw 2006	Obstretrics & Gynecology	The Netherlands	Cohort study	Pre-eclampsia; HELLP Syndrome	0.5	66	Unknown	Unknown	31	31	Unknown
Blaauw 2014	Acta Obstetricia et Gynecolologica Scandinavica	The Netherlands	Cohort study	Pre-eclampsia	4.7	33	100% Caucasian	Unknown	33	Unknown	Unknown
Bokslag 2017	American Journal of Obstetrics and Gynecology	The Netherlands	Cross sectional study	Pre-eclampsia	13.1	187	86.3% Caucasian	30.9	44	30.5	1136
Breetveld 2015	British Journal of Obstetrics and Gynaecology	The Netherlands	Cross sectional study	Pre-eclampsia	5.4	165	Unknown	Unknown	36	33.3	1800
Catov 2013	Hypertension	United States of America	Cohort study	Preterm Birth	20	916	62.39% Black	29.89	44.1	Unknown	Unknown
Catov 2013	Journal of Women's Health	United States of America	Cross sectional study	Preterm Birth	8.5	487	26% African-American, 74% Caucasian	30.5	39	34	Unknown
Catov 2019	Journal of Women's Health	United States of America	Cross sectional study	Preterm Birth	11.1	605	32.9% African-American	26.4	37.5	Unknown	Unknown
Chambers 2001	Journal of the American Medical Association	England	Case control study	Pre-eclampsia	3	161	Unknown	Unknown	34	Unknown	Unknown
Christensen 2016	Pregnancy Hypertension	Denmark	Cohort study	Pre-eclampsia	10.28	42	Unknown	Unknown	40.75	37.4	2917

Ciftci 2014	Hypertension	Turkey	Cohort study	Pre-eclampsia	5	84	Unknown	Unknown	33.28	Unknown	Unknown
Cortes 2018	Journal of the American Heart Association	United States of America	Cross sectional study	Small for Gestational Age; Preterm Birth; Stillbirth	14.3	1120	50% Caucasian, 29% Black, 10.5% Hispanic, 10.5% Chinese PTB; 28.8% Caucasian, 49.2% Black, 8.5% Hispanic, 13.6% Chinese SGA; 50% Caucasian, 36.4% Black, 9.1% Hispanic, 4.6% Chinese Stillbirth	24.4 PTB; 22.7 SGA; 24.1 Stillbirth	60.0 PTB; 60.1 SGA; 59.7 Stillbirth	Unknown	Unknown
Costacou 2008	Preventative Cardiology	United States of America	Cohort study	Gestational Diabetes; Pre-eclampsia	2.3 PE; 2.1 GDM	117	2.3% African-American PE; 2,1% African-American GDM	29.0 PE; 28.9 GDM	Unknown	Unknown	Unknown
DeHavenon 2021	Journal of the American Medical Association	United States of America	Cross sectional study	Pre-eclampsia	35	1435	100% Caucasian	Unknown	44.4, 77.5	Unknown	Unknown
DiCianni 2007	Diabetes/Metablism Research and Reviews	Italy	Cohort study	Gestational Diabetes	1.33	264	Unknown	Unknown	34.7	Unknown	Unknown
Dorans 2022	Nutrition, Metabolism & Cardiovascular Diseases	United States of America	Cross sectional study	Gestational Diabetes	8.6	3537	54.6% Non-Hispanic White, 11.2% Non-Hispanic Black, 23.8% Hispanic	23	35.9	Unknown	Unknown
Drost 2012	European Journal of Preventive Cardiology	The Netherlands	Cross sectional study	Pre-eclampsia	9.1	671	Unknown	29.8	38.9	Unknown	1438
Ernawati 2019	Biochemical and Cellular Archives	Indonesia	Cohort study	Pre-eclampsia	5.3	42	100% Asian	30	35	35.6	2396.705
Escouto 2018	Pregnancy Hypertension	United Kingdom	Cohort study	Pre-eclampsia	0.14	477	82% Caucasian, 10% Black, 6% Asian, 2%Other PE; 90% Caucasian, 10% Black Superimposed PE	Unknown	29	38.15	2722.5
Evans 2011	Hypertension	United States of America	Cohort study	Pre-eclampsia	1.33	68	83.3% Caucasian, 16.4% Black	Unknown	28.1	Unknown	Unknown
Fakhrzadeh 2011	Journal of Diabetes and Metabolic Disorders	Iran	Cohort study	Gestational Diabetes	5	40	Unknown	Unknown	31.36	Unknown	Unknown
Fatma 2017	Journal of the Association of Physicians of India	India	Cross sectional study	Pre-eclampsia	8	100	Unknown	Unknown	34.82	Unknown	Unknown
Forest 2005	American Journal of Obstetrics and Gynecology	Canada	Cross sectional study	Pre-eclampsia	7.8	336	100% Caucasian	27.4	35.5	38.3	3120
Fraser 2022	Journal of the American Heart Association	Norway	Cohort study	Pre-eclampsia; Fetal Growth Restriction; Preterm Birth	25	13861	Unknown	23 PE; 22 PTB; 22 FGR	49 PE; 49 PTB; 49 FGR	Unknown	Unknown
Freire 2012	Cardiovascular Diabetology	Brazil	Cross sectional study	Gestational Diabetes	2.7	169	Unknown	Unknown	36.42	Unknown	Unknown
Garrido-Gimenez 2020	Hypertension	Spain	Cohort study	Pre-eclampsia	12.7	64	67.4% Caucasian	30	42	33.5	1415; 1 percentile
Ghossein-Doha 2016	Ultrasound in Obstetetrics & Gynecology	The Netherlands	Cross sectional study	Pre-eclampsia	4.8	148	100% Caucasian	Unknown	36	34	1836
Gladstone 2019	Journal of Obstetics and Gynaecology Canada	Canada	Cohort study	Pre-eclampsia; HELLP Syndrome	0.5	889	89.4% Caucasian, 10.6% Other	Unknown	31	Unknown	3040
Goynumer 2013	Journal of Clinical Ultrasound	Turkey	Cohort study	Pre-eclampsia	0.75	76	Unknown	Unknown	30.94	32.68	2040
Gumus 2012	Blood Coagluation & Fibrinolysis	Turkey	Case control study	Gestational Diabetes	1	70	Unknown	Unknown	32.9	Unknown	Unknown
Gunderson 2014	Journal of the American Heart Association	United States of America	Cohort study	Gestational Diabetes	20	898	43% Black	24.7	44.8	Unknown	Unknown

Haug 2018	Journal of the American Heart Association	Norway	Cohort study	Pre-eclampsia	25.4	23878	97% Caucasian	24	31	Unknown	Unknown
Hauge 2022	Journal of the American College of Cardiology	Denmark	Cross sectional study	Pre-eclampsia; HELLP Syndrome	14.5	1507	93.6% European	Unknown	46.8	Unknown	Unknown
Haukkamaa 2009	Cerebrovascular Diseases	Finland	Cross sectional study	Pre-eclampsia	32	767	Unknown	Unknown	57.2	Unknown	Unknown
Heidema 2015	European Journal of Obstetrics & Gynecology and Reproductive Biology	The Netherlands	Case control study	Pre-eclampsia	1.06	120	Unknown	31.2	Unknown	32.4	1645
Heitritter 2005	The Journal of Clinical Endocrinology & Metabolism	United States of America	Case control study	Gestational Diabetes	4	46	Mostly Caucasian	Unknown	37	Unknown	Unknown
Hooijschuur 2015	American Journal of Obstetrics and Gynecology	The Netherlands	Cohort study	Pre-eclampsia; Small for Gestational Age	0.58 SGA; 0.83 PE; 0.75 PE with SGA	1303	Unknown	31.4 SGA; 31.8 PE; 31.2 PE with SGA	Unknown	32.6 SGA; 34.0 PE; 31.5 PE with SGA	1258 SGA; 2118 PE; 1210 PE with SGA
Hromadnikova 2019	Journal of Clinical Medicine	Czech republic	Cohort study	Pre-eclampsia; Fetal Growth Restriction	5.33 PE; 5.05 FGR	276	100% Caucasian	32.54 PE; 31.91 FGR	38.05 PE; 37.0 FGR	35.97 PE; 35.65 FGR	2416.74 PE; 1910.00 FGR
Hu 1998	British Journal of Obstetrics and Gynaecology	Sweden	Cross sectional study	Gestational Diabetes	3	37	Unknown	Unknown	36	Unknown	Unknown
Ijas 2013	European Journal of Endocrinology	Finland	Cross sectional study	Gestational Diabetes	18.6	116	Unknown	Unknown	52.8	Unknown	Unknown
Janmohamed 2015	Journal of Obstetics and Gynaecology Canada	Canada	Cohort study	Pre-eclampsia; HELLP Syndrome	0.5	21	Unknown	29.1	Unknown	31.8	Unknown
Kim 2008	Obstretrics & Gynecology	United States of America	Cross sectional study	Gestational Diabetes	7	4631	55% Non-Hispanic White, 19% African American, 17% Mexican American, 9% Other	Unknown	Unknown	Unknown	Unknown
Kim 2020	BMC Pregnancy and Childbirth	South Korea	Cross sectional study	Pre-eclampsia	0.5	73	Unknown	34.8	Unknown	33.7	1790
King 2009	Research in Nursing & Health	United States of America	Case control study	Gestational Diabetes	5	40	Unknown	Unknown	44.8	Unknown	3450.2
Ko 1999	Australian and New Zealand Journal of Obstetrics and Gynaecology	Hong Kong	Cohort study	Gestational Diabetes	0.12	1232	100% Chinese	Unknown	34	Unknown	Unknown
Kosinski 2021	British Medical Journal: Open Diabetes Research & Care	Switzerland	Cohort study	Gestational Diabetes	1	784	33% Swiss, 32% Eurpoean, 18.5% African 13% Asian, 1.2% Latin American	33.5	Unknown	Unknown	Unknown
Kul 2021	Microvascular Research	Turkey	Cohort study	Gestational Diabetes; Pre-eclampsia	1	142	Unknown	Unknown	31.0 PE; 35.0 GDM; 33.5 PE with GDM	Unknown	Unknown
Lekva 2015	Public Library of Science One	Norway	Cohort study	Gestational Diabetes	5	300	Unknown	33.6	38.9	40.4	3832
Magnussen 2009	Obstretrics & Gynecology	Norway	Cross sectional study	Pre-eclampsia	16.3	15064	Mostly Caucasian	Unknown	40.1	Unknown	Unknown
Mahendru 2013	The Journal of Maternal-Fetal and Neonatal Medicine	United Kingdom	Cohort study	Pre-eclampsia; Intrauterine Growth Restriction	3.33	74	Unknown	Unknown	37	36	2270
Mai 2014	Gynecological Endocrinology	China	Case control study	Gestational Diabetes	2.5	270	Unknown	Unknown	33.1	Unknown	Unknown

Mangos 2012	Journal of Hypertension	Australia	Cross sectional study	Pre-eclampsia	3.8	101	Unknown	Unknown	37	Unknown	Unknown
Manten 2007	Hypertension in Pregnancy	The Netherlands	Cohort study	Pre-eclampsia; Intrauterine Growth Restriction	0.82 PE; 0.77 IUGR	368	100% Caucasian	31	32	31 PE; 30 IUGR	1316 PE; 972 IUGR
Markovitz 2021	Acta Obstetricia et Gynecolologica Scandinavica	Norway	Cohort study	Preterm Birth	10	19806	Unknown	22	Unknown	Unknown	Unknown
Martillotti 2013	Hypertension	Switzerland	Cross sectional study	Pre-eclampsia	8	40	76% Caucasian, 24% African	Unknown	39.8	29.1	Unknown
McDonald 2013	Atherosclerosis	Canada	Cohort study	Pre-eclampsia	20	327	91.7% European	Unknown	49	Unknown	Unknown
Meyers-Seifer 1996	Diabetes Care	United States of America	Cohort study	Gestational Diabetes	5.5	104	Unknown	Unknown	36.9	Unknown	3700
Moe 2020	European Journal of Preventive Cardiology	Norway	Cohort study	Gestational Diabetes; Pre-eclampsia	1	235	3.8% Non-White Late PE; 6.8% Non-White Early PE; 32% Non-White GDM	Unknown	35	Unknown	Unknown
Moe 2020	Pregnancy Hypertension	Norway	Cohort study	Pre-eclampsia	1	221	96.3% Caucasian	Unknown	34.3	31	Unknown
Murphy 2013	Pregnancy Hypertension	Canada	Cohort study	Pre-eclampsia	0.5	30	Unknown	31.5	Unknown	35.9	Unknown
Murphy 2014	Physiological Reports	Canada	Cohort study	Pre-eclampsia	0.5	63	Unknown	32.1	Unknown	36	Unknown
Murphy 2015	Pregnancy Hypertension	Canada	Cohort study	Pre-eclampsia	0.5	63	85.7% Caucasian	Unknown	33.2	35.9	Unknown
Nouhjah 2017	Iranian Red Crescent Medical Journal	Iran	Cohort study	Gestational Diabetes	0.15	262	35.8% Fars, 18.8% Lor, 45.5% Arab	Unknown	29.68	Unknown	Unknown
Ostlund 2013	Hypertension Research	Sweeden	Cohort study	Pre-eclampsia	11	31	Unknown	Unknown	39.4	35	2557
Paradisi 2011	The Journal of Obstetrics and Gynaecology Research	Italy	Cohort study	Small for Gestational Age	2.33	31	Unknown	Unknown	34.7	38	2181; 8.4 percentile
Perera 2019	Women & Health	United States of America	Cohort study	Gestational Diabetes	9	8262	100% Latina/Hispanic	30.1	39.1	Unknown	Unknown
Retnakaran 2021	Diabetes, Obesity & Metabolism	Canada	Case control study	Gestational Diabetes	2	39581	77.5% General Population, 12.9% Chinese, 9.7% South Asian	Unknown	33.1	Unknown	Unknown
Roca-Rodriguez 2012	Diabetes/Metabolism Research and Reviews	Spain	Case control study	Gestational Diabetes	1	62	Unknown	Unknown	33.8	Unknown	Unknown
Romagnuolo 2016	Fertility and Sterility	Italy	Cohort study	Pre-eclampsia; Small for Gestational Age; Stillbirth	0.23	630	Unknown	Unknown	35	Unknown	Unknown
Romundstad 2010	Circulation	Norway	Cross sectional study	Pre-eclampsia	11	3225	Unknown	Unknown	38.5	Unknown	Unknown
Sattar 2003	Hypertension	Unknown	Cross sectional study	Pre-eclampsia	20	80	Unknown	24	43	36	18 percentile
Schreurs 2015	British Journal of Obstetrics and Gynaecology	The Netherlands	Cohort study	Pre-eclampsia	0.92	786	Unknown	31 PE; 30.3 Eclampsia	Unknown	33 PE; 34 Eclampsia	1737 PE; 2218 Eclampsia; 28 percentile PE; 35 percentile Eclampsia
Shi 2018	Anatolian Journal of Cardiology	China	Cohort study	Preterm Birth	15	1974	100% Kailuan	Unknown	40.84	Unknown	Unknown
Smith 2009	American Journal of Obstetrics and Gynecology	Canada	Cohort study	Pre-eclampsia	1	180	91.4% Caucasian	30.5	Unknown	35.6	2496.3
Smith 2012	Journal of Obstetics and Gynaecology Canada	Canada	Cohort study	Pre-eclampsia	1	217	96% Caucasian	30.3	Unknown	Unknown	Unknown

Sokup 2014	Gynecological Endocrinology	Poland	Cross sectional study	Gestational Diabetes	1.5	124	100% Caucasian	30	Unknown	Unknown	Unknown
Souwer 2011	Acta Obstetricia et Gynecolologica Scandinavica	The Netherlands	Case control study	Pre-eclampsia	4.6	30	Unknown	Unknown	33	31	1100
Spaan 2010	Microvascular Research	The Netherlands	Cross sectional study	Pre-eclampsia	23	51	100% Caucasian	Unknown	49	35	1801
Tanz 2021	Journal of Women's Health	United States of America	Cohort study	Preterm Birth	25	116,429	Unknown	Unknown	47	Unknown	Unknown
vanRijn 2013	Obstretrics & Gynecology	The Netherlands	Cohort study	Pre-eclampsia	0.5	617	99.6% Caucasian	Unknown	30.5	29.8	1082
vanRijn 2014	Hypertension	The Netherlands	Cohort study	Pre-eclampsia	0.67	150	Unknown	Unknown	30.5	Unknown	Unknown
Veerbeek 2013	Hypertension	The Netherlands	Cohort study	Placental Abruption	0.67	154	97.3% Caucasian	Unknown	30.9	29.6	1024
Veerbeek 2015	Hypertension	The Netherlands	Cohort study	Pre-eclampsia	0.67	748	94.9% Caucasian	Unknown	33	34.235	2094
Veerbeek 2016	Hypertension	The Netherlands	Cohort study	Pre-eclampsia	0.67	58	86.2% Caucasian	Unknown	30.5	30.3	1135
Verma 2002	The Journal of Clinical Endocrinology & Metabolism	United States of America	Cross sectional study	Gestational Diabetes	11	207	92% Caucasian, 3% Black, 5% Hispanic	30.1	Unknown	Unknown	Unknown
Vilmi-Kerälä 2015	Diabetology & Metabolic Syndrome	Finland	Cross sectional study	Gestational Diabetes	3.7	240	Unknown		25.8	39.6	3633
Wen 2019	Journal of Clinical Lipidology	Canada	Case control study	Pre-eclampsia	1.5	27300	Unknown	31.3	Unknown	35.5	Unknown
White 2016	American Journal of Obstetrics and Gynecology	United States of America	Cohort study	Pre-eclampsia	34.9	80	100% Caucasian	24.2	59.4	Unknown	Unknown
Wu 2019	Journal of Vascular Health	China	Cross sectional study	Pre-eclampsia	4.92	168	Unknown	Unknown	35.6	Unknown	Unknown
Xuereb 2019	Canadian Journal of Diabetes	Malta	Cohort study	Gestational Diabetes	8	203	Unknown	Unknown	38.65	Unknown	Unknown
Yinon 2010	Circulation	Canada	Cross sectional study	Pre-eclampsia; Intrauterine Growth Restriction	2	49	78% Caucasian PE; 56% Caucasian IUGR	Unknown	35.5 PE; 33 IUGR	Unknown	Unknown
Zajdenverg 2014	Diabetology & Metabolic Syndrome	Brazil	Cross sectional study	Gestational Diabetes	1	45	40% Caucasian	Unknown	33.5	Unknown	Unknown
Zaza 2021	European Journal of Obstetrics & Gynecology and Reproductive Biology	Canada	Cohort study	Pre-eclampsia	0.5	186	91.2% Caucasian	31.4	Unknown	Unknown	Unknown

S3. Embase Search

Concept #	Search Terms
Cardiovascular Disease	
1	exp Cardiovascular System/ or exp Cardiovascular Diseases/ or exp Cardiology/ or exp Heart Disease Risk Factors/ or exp Vascular Diseases/ or exp Aortic Diseases/ or exp Cerebrovascular Disorders/ or exp Cardiomyopathy/
2	(cardiac* or heart* or cardiolog* or cardiovasc* or coronary or vascular* or cerebrovasc* or arterial* or artery or arteries or aorta or aortic or vein* or venous* or valve or valvular or ventric* or arrhythm* or cardiomyopath* or infarct* or stroke* or hypertens* or endocardi* or myocardi* or pericardi* or angiog* or angioc* or Electrocardiograph* or Electrocardiogram* or ECG* or EKG* or holter*).ti,ab.
Pregnant Population	
3	exp Pregnancy/ or Pregnant Women/ or exp Obstetrics/ or exp Labor, Obstetric/
4	(pregnan* or obstetric* or postpartum* or postnatal* or labor* or deliver* or maternal*).ti,ab.
5	((post adj3 natal*) or (post adj3 partum*) or (labor adj3 delivery)).ti,ab.
Placental Diseases	
6	exp Placenta Diseases/ or exp Pregnancy Complications/ or exp Obstetric Labor Complications/
7	((pregnanc* adj3 complicat*) or placenta mediated disease* or (placenta* adj disease*) or (pregnanc* adj3 disease*) or (placenta* adj disorder) or labor complicat* or (obstetric* adj3 complicat*)).ti,ab.
8	exp diabetes, gestational/
9	gestational diabetes.ti,ab.
10	exp Hypertension, Pregnancy-Induced/
11	(eclamp* or pre eclamp* or preeclamp* or pre-eclamp* or hellp syndrome* or hellp* or tox?emi*).ti,ab.
12	Abruptio Placentae/
13	(placent* adj2 abruption).ti,ab.
14	exp Infant, Low Birth Weight/
15	Fetal Growth Retardation/
16	(intrauterine growth restriction* or intra-uterine growth restriction* or IUGR* or FGR* or low birth weight or small for gestational age).ti,ab.
17	((fetal or foetal or fetus or foetus) adj3 (restriction* or retard*)).ti,ab.
18	exp Fetal Death/
19	intrauterine f?etus demise.ti,ab.
20	(IUFD* or neonatal death* or stillbirth* or still birth* or stillborn* or still born* or ((fetal or foetal or fetus or foetus) adj3 death*)).ti,ab.
21	intrauterine f?etal demise.ti,ab.
22	exp Infant, Premature/
23	(prematur* or preterm* or pre term*).ti,ab.
Study Methodology	
24	exp Cohort Studies/ or exp Case Control Studies/ or exp Risk Factors/
25	(risk factor* or risk assessment or risk assess* or risk equation or risk calc* or risk scor* or risk predict* or risk factor calc* or prediction model* or scoring* method* or scoring* scheme* or risk estim*).ti,ab.
26	(framingham equation* or framingham estim* or framingham algorithm or framingham guideline* or framingham risk or framingham score or framingham model* or score risk* or score algorithm* or arterial assess* or arterial health assess* or hemodynamic* or arterial tonometry or arterial stiffness or hemodynamic load* or vascular doppler* or vascular doppler sonography).ti,ab.
Summation	
27	1 or 2
28	3 or 4 or 5
29	or/6-23
30	24 and (25 or 26)
31	27 and 28 and 29 and 30
32	31 not (exp animals/ not humans/)
33	32 not (exp Systematic Review/ or exp Meta-Analysis/ or exp Review/)
34	33 not (child* or offspring*).ti,ab,kw.
35	34 not (exp child/ or adult children/ or exp male/)
36	limit 35 to (english language or french)

S4. CINHAL Search

Concept #	Search Terms
Cardiovascular Disease	
1	MH Cardiovascular System+ or MH Cardiovascular Diseases+ or MH Cardiology+ or MH Heart Disease Risk Factors+ or MH Vascular Diseases+ or MH Aortic Diseases+ or MH Cerebrovascular Disorders+ or MH Cardiomyopathy+
2	TI (cardiac* or heart* or cardiolog* or cardiovasc* or coronary or vascular* or cerebrovasc* or arterial* or artery or arteries or aorta or aortic or vein* or venous* or valve or valvular or ventric* or arrhythm* or cardiomyopath* or infarct* or stroke* or hypertens* or endocardi* or myocardi* or pericardi* or angiog* or angioc* or Electrocardiograph* or Electrocardiogram* or ECG* or EKG* or holter* or CVD*) OR AB (cardiac* or heart* or cardiolog* or cardiovasc* or coronary or vascular* or cerebrovasc* or arterial* or artery or arteries or aorta or aortic or vein* or venous* or valve or valvular or ventric* or arrhythm* or cardiomyopath* or infarct* or stroke* or hypertens* or endocardi* or myocardi* or pericardi* or angiog* or angioc* or Electrocardiograph* or Electrocardiogram* or ECG* or EKG* or holter* or CVD*)
Pregnant Population	
3	MH Pregnancy+ OR MH "Pregnant Women+ OR MH Obstetrics+ OR MH Labor, Obstetric+
4	TI (pregnan* or obstetric* or postpartum* or postnatal*or (post N natal*) or (post N partum*) or (labor N3 delivery) or labor* or deliver* or maternal*) OR AB (pregnan* or obstetric* or postpartum* or postnatal*or (post N natal*) or (post N partum*) or (labor N3 delivery) or labor* or deliver* or maternal*)
Placental Diseases	
5	MH Placenta Diseases+ OR MH Pregnancy Complications+ OR MH Obstetric Labor Complications+
6	TI (pregnanc* N3 complicat* or placenta mediated disease* or placenta* N disease* or pregnanc* N3 disease* or placenta* N disorder or labor complicat* or obstetric* N3 complicat*) OR AB (pregnanc* N3 complicat* or placenta mediated disease* or placenta* N disease* or pregnanc* N3 disease* or placenta* N disorder or labor complicat* or obstetric* N3 complicat*)
7	MH Diabetes Mellitus, gestational
8	TI gestational diabetes OR AB gestational diabetes
9	MH Pregnancy-Induced Hypertension+
10	TI (eclamp* or pre eclamp* or preeclamp* or pre-eclamp* or hellp syndrome* or hellp* or toxemi* or toxaemi*) OR AB (eclamp* or pre eclamp* or preeclamp* or pre-eclamp* or hellp syndrome* or hellp* or toxemi* or toxaemi*)
11	MH Abruptio Placentae
12	TI (placent* N2 abruption) OR AB (placent* N2 abruption)
13	MH Infant, Low Birth Weight+
14	MH Fetal Growth Retardation
15	TI (intrauterine growth restriction* or intra-uterine growth restriction* or IUGR* Or FGR* or low birth weight or small for gestational age ((fetal or foetal or fetus or foetus) N3 (restriction* or retard*))) OR AB (intrauterine growth restriction* or intra-uterine growth restriction* or IUGR* Or FGR* or low birth weight or small for gestational age ((fetal or foetal or fetus or foetus) N3 (restriction* or retard*)))
16	MH Perinatal Death+
17	TI (intrauterine fetal demise or IUFD* or intrauterine foetal demise or neonatal death* or stillbirth* or still birth* or stillborn* or still born* or ((fetal or foetal or fetus or foetus) N3 (death*))) OR AB (intrauterine fetal demise or IUFD* or intrauterine foetal demise or neonatal death* or stillbirth* or still birth* or stillborn* or still born* or ((fetal or foetal or fetus or foetus) N3 (death*)))
18	MH Infant, Premature+
19	TI (prematur* or preterm* or pre term*) OR AB (prematur* or preterm* or pre term*)
Study Methodology	
20	MH Cohort Studies+ OR MH Case Control Studies+ or MH Risk Factors+
21	TI (risk factor* or risk assessment or risk assess* or risk equation or risk calc* or risk scor* or risk predict* or risk factor calc* or prediction model* or scoring* method* or scoring* scheme* or risk estim*) OR AB (risk factor* or risk assessment or risk assess* or risk equation or risk calc* or risk scor* or risk predict* or risk factor calc* or prediction model* or scoring* method* or scoring* scheme* or risk estim*)
22	TI (framingham equation* or framingham estim* or framingham algorithm or framingham guideline* or framingham risk or framingham score or framingham model* or score risk* or score algorithm* or arterial assess* or arterial health assess* or hemodynamic* or arterial tonometry or arterial stiffness or hemodynamic load* or vascular doppler* or vascular doppler sonography) OR AB (framingham equation* or framingham estim* or framingham algorithm or framingham guideline* or framingham risk or framingham score or framingham model* or score risk* or score algorithm* or arterial assess* or arterial health assess* or hemodynamic* or arterial tonometry or arterial stiffness or hemodynamic load* or vascular doppler* or vascular doppler sonography)
Summation	
23	S1 OR S2
24	S3 OR S4
25	S5 OR S6 OR S7 OR S8 OR S9 OR S10 OR S11 OR S12 OR S13 OR S14 OR S15 OR S16 OR S17 OR S18 OR S19
26	S20 AND (S21 OR S22)

27	S23 AND S24 AND S25 AND S26
28	S27 NOT (MH Review+ OR MH meta-analysis+ OR MH systematic review+)
29	S28 NOT (TI(child* or offspring*) OR AB (child* or offspring*))
30	S29 NOT(MH child+ or MH adult children+ or MH male+)

S5. Scopus Search

((TITLE-ABS (cardiac* OR heart* OR cardiolog* OR cardiovasc* OR coronary OR vascular* OR cerebrovasc* OR arterial* OR artery OR arteries OR aorta OR aortic OR vein* OR venous* OR valve OR valvular OR ventric* OR arrhythm* OR cardiomyopath* OR infarct* OR stroke* OR hypertens* OR endocardi* OR myocardi* OR pericardi* OR angiog* OR angioc* OR electrocardiograph* OR electrocardiogram* OR ecg* OR ekg* OR holter* OR cvd*))) AND (TITLE-ABS (pregnan* OR obstetric* OR postpartum* OR postnatal* OR (post W/ natal*) OR (post W/ partum*) OR (labor W/3 delivery) OR labor* OR deliver* OR maternal*)) AND (TITLE-ABS ((pregnanc* W/3 complicat*) OR ("placenta mediated disease*") OR (placenta* W/ disease*) OR (pregnanc* W/3 disease*) OR (placenta* W/ disorder) OR ("labor complicat*") OR (obstetric* W/3 complicat*))) OR (TITLE-ABS ("gestational diabetes")) OR (TITLE-ABS (eclamp* OR "pre eclamp*" OR preeclamp* OR pre-eclamp* OR "hellp syndrome*" OR hellp* OR toxemi* AND toxaemi*)) OR (TITLE-ABS (placent* W/2 abruption)) OR (TITLE-ABS ("intrauterine growth restriction*" OR "intra-uterine growth restriction*" OR iugr* OR fgr* OR "low birth weight" OR "small for gestational age")) OR (TITLE-ABS ((fetal OR foetal OR fetus OR foetus) W/3 (restriction* OR retard*))) OR (TITLE-ABS ("intrauterine fetal demise" OR "intrauterine feotal demise" OR iufd*)) OR (TITLE-ABS (prematur* OR preterm* OR "pre term*")) AND (("Cohort Studies" OR "Case Control Studies" OR "Risk Factors") AND ((TITLE-ABS ("risk factor*" OR "risk assessment" OR "risk assess*" OR "risk equation" OR "risk calc*" OR "risk scor*" OR "risk predict*" OR "risk factor calc*" OR "prediction model*" OR "scoring* method*" OR "scoring* scheme*" OR "risk estim*")) OR (TITLE-ABS ("framingham equation*" OR "framingham estim*" OR "framingham algorithm" OR " guideline*" OR "framingham risk" OR "framingham score" OR "framingham model*" OR "score risk*" OR "score algorithm*" OR "arterial assess*" OR "arterial health assess*" OR hemodynamic* OR "arterial tonometry" OR "arterial stiffness" OR "hemodynamic load*" OR "vascular doppler*" OR "vascular doppler sonography")))) AND NOT (TITLE-ABS (child* OR offspring*)) AND (LIMIT-TO (DOCTYPE , "ar")) AND (LIMIT-TO (LANGUAGE , "English") OR LIMIT-TO (LANGUAGE , "French")) AND (LIMIT-TO (EXACTKEYWORD , "Human"))

S6. Modified Newcastle-Ottawa Quality Scale

1. Method of exposure ascertainment
    a) Secure record (medical chart and reference to diagnostic criteria) **
    b) Self-report or record linkage *
    c) No description
2A. Case-Control Studies: Representativeness of the cases
    a) Consecutive or obviously representative series of cases *
    b) Potential for selection bias or not stated
2B. Cohort Studies: Representativeness of the exposed cohort
    a) Truly representative of the average in the community **
    b) Somewhat representative of the average in the community *
    c) Selected group of users
    d) No description of the deviation of the cohort
2C. Cross-Sectional Studies: Representativeness of the sample
    a) Truly representative of the average in the target population** (all subjects or random sampling)
    b) Somewhat representative of the average in the target population * (non-random sampling)
    c) Selected group of users
    d) No description of the sampling strategy
3. Demonstration that the outcome of interest was not present at the start of the study
    a) Yes, exclude participants with chronic
    b) No description
4. Definition of controls

a) Confirmation of no history of disease (medical record) **
b) Self-report or record linkage *
c) No description
d) No controls

5. Selection of controls
a) Drawn from the same community as cases *
b) Drawn from a different source
c) No description of the deviation of the controls

6. Comparability of cases and controls on the basis of design or analysis
a) Study controls for any additional confounding variables **
b) Match controls by BMI or age or another variable *
c) Do not control, just complete a direct comparison

7. Sample size
a) Justified and satisfactory *
b) Not justified

8A. Case-Control Studies: Non-response rate
a) Same rate for cases and controls *
b) Non respondents described or different rate
c) No description of the response rate or the characteristics of the responders and the non-responders

8B. Cohort Studies: Adequacy of follow-up of cohorts
a) Complete follow up **
b) Subjects lost to follow up unlikely to introduce bias (80%) or description provided of those lost *
c) Follow up rate< 80% and no description of those lost
d) No statement

8C. Cross-Sectional Studies: Non-respondents
a) Comparability between respondents and non-respondents characteristics is established, and the response rate is satisfactory *
b) The response rate is unsatisfactory, or the comparability between respondents and non-respondents is unsatisfactory
c) No description of the response rate or the characteristics of the responders and the non-responders

9) Was follow-up long enough for outcomes to occur?
a) Yes-over 1 year postpartum **
b) Some between 6 months- 1 year *
c) No

10) Ascertainment of outcome
a) Blinded CVD risk assessment **
b) Secure health record *
c) Self report of biochemical measures
d) No description

11. Same method of ascertainment for cases and controls
a) Yes *

b) No

12) Appropriate statistical tests used

a) The statistical test used to analyze the data is clearly described and appropriate, and the measurement of the association is presented, including confidence intervals and the probability level (p value). *

b) The statistical test is not appropriate, not described, or incomplete.

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Chapter 3

Placental pathology as a tool to identify women for postpartum cardiovascular risk screening following preeclampsia: a preliminary investigation

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Placental Pathology as a Tool to Identify Women for Postpartum Cardiovascular Risk Screening following Preeclampsia: A Preliminary Investigation

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Abstract: Preeclampsia (PE) is associated with an increased risk of cardiovascular disease (CVD) in later life. Postpartum cardiovascular risk screening could identify patients who would benefit most from early intervention and lifestyle modification. However, there are no readily available methods to identify these high-risk women. We propose that placental lesions may be useful in this regard. Here, we determine the association between placental lesions and lifetime CVD risk assessed 6 months following PE. Placentas from 85 PE women were evaluated for histopathological lesions. At 6 months postpartum, a lifetime cardiovascular risk score was calculated. Placental lesions were compared between CVD risk groups and the association was assessed using odds ratios. Multivariable logistic regression was used to develop prediction models for CVD risk with placental pathology. Placentas from high-risk women had more severe lesions of maternal vascular malperfusion (MVM) and resulted in a 3-fold increased risk of screening as high-risk for CVD (OR 3.10 (1.20–7.92)) compared to women without these lesions. MVM lesion severity was moderately predictive of high-risk screening (AUC 0.63 (0.51, 0.75); sensitivity 71.8% (54.6, 84.4); specificity 54.7% (41.5, 67.3)). When clinical parameters were added, the model's predictive performance improved (AUC 0.73 (0.62, 0.84); sensitivity 78.4% (65.4, 87.5); specificity 51.6% (34.8, 68.0)). The results suggest that placenta pathology may provide a unique modality to identify women for cardiovascular screening.

Keywords: preeclampsia; placenta; histopathology; cardiovascular disease; cardiovascular risk; postpartum screening

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1. Introduction

Preeclampsia (PE) is a life-threatening hypertensive disorder of pregnancy, affecting 5–7% of pregnancies worldwide [1]. Importantly, PE is a significant risk indicator for cardiovascular disease (CVD) in later life. Women diagnosed with PE have a ~4-fold increased risk of hypertension and a ~2-fold increased risk of ischemic heart disease and stroke compared to women with uncomplicated pregnancies [2–5]. Moreover, evidence suggests that women who develop severe PE during pregnancy are at the highest risk of these outcomes [4,6–8]. Alarmingly, studies show that the onset of CVD and CVD-related death occur at much younger ages than the general female population [6–9]. The link(s) between PE and cardiovascular risk are not fully understood, but PE may indicate the presence of underlying, often undiagnosed, cardiovascular risk factors (CVRs) [10,11].

Moreover, underlying CVRs may directly contribute to placental dysfunction associated with PE; however, this relationship has yet to be fully elucidated [12,13].

Histopathological lesions of maternal vascular malperfusion (MVM) are commonly observed in placentas from PE pregnancies, particularly in cases of severe, early-onset disease [14–16]. These lesions, including increased syncytial knots and accelerated villous maturation, are believed to reflect placental hypoxia and oxidative stress arising from incomplete uterine artery modeling and abnormal uteroplacental blood flow [1,17,18]. Although common, MVM lesions are not observed in all PE cases, and a proportion of PE placentas are histologically normal or exhibit other pathology such as chronic inflammation [19,20]. Recent population-based studies have shown an association between placental lesions and future maternal health [21–26]. Catov et al. observed altered cardiometabolic profiles in women with preterm birth and lesions of MVM compared to women with term deliveries [21,26]. Additionally, women with preterm birth and co-morbid placental pathologies (MVM, inflammatory lesions) had the most severe atherogenic profiles [21]. More recently, Catov et al. demonstrated that MVM lesions are associated with vascular impairments 8–10 years after pregnancy [26]. While the mechanisms underlying these associations have yet to be fully elucidated, collectively, these studies provide strong evidence that the placenta and its pathology may provide a snapshot into future maternal cardio-metabolic health [26,27].

To reduce the burden of CVD on PE women, specialized postpartum cardiovascular health clinics are being established across North America to screen women for CVRs and initiate postpartum CVD prevention, including pharmaceutical and lifestyle interventions [28–30]. However, these clinics are resource-intensive, and, thus, follow-up of all PE women is prohibitive. Moreover, a proportion of PE women will remain at low risk for CVD postpartum and not require follow-up. As placental pathology is inexpensive and readily available, it may offer a unique modality to identify PE women for CVR screening. Here, we investigate the association between placental pathology and lifetime CVD risk in postpartum women following PE.

2. Materials and Methods

In this study, a cohort of women diagnosed with PE who underwent cardiovascular health screening at 6 months postpartum was assembled from two study sites (Kingston and Ottawa, ON, Canada).

2.1. Recruitment at the Kingston Site

In Kingston, women who develop a pregnancy complication are referred to the Maternal Health Clinic (Kingston General Hospital, Kingston, ON, Canada) for postpartum cardiovascular health screening at 6 months postpartum as part of routine standard of care. Assessments and evaluations, including the calculation of a lifetime cardiovascular risk score conducted at the Maternal Health Clinic, have been previously described [29]. From this clinic, we recruited eligible study participants at the time of their 6-month postpartum visit (between 2011 and 2017). Women diagnosed with PE who had placenta pathology performed at delivery of the index pregnancy (PE diagnosis) were approached to participate in the study.

2.2. Recruitment at the Ottawa Site

In Ottawa, women were recruited to participate in the study as part of the DREAM Study research protocol designed to emulate the Maternal Health Clinic. Women diagnosed with PE prior to delivery were recruited from inpatient services at the Ottawa Hospital General Campus (Ottawa, ON, Canada) between 2013 and 2018. Placentas from each participant were sent to Anatomical Pathology at the Children's Hospital of Eastern Ontario (Ottawa, ON, Canada). Six months after delivery, participants returned to the Ottawa Hospital for cardiovascular health screening performed by the research study nurse. The assessments performed at this study visit were identical to those performed at the Maternal

Health Clinic, and a lifetime cardiovascular risk score was calculated for each participant, as described previously [29]. At both sites, all women provided written informed consent to participate in the study.

2.3. Inclusion and Exclusion Criteria

PE was defined according to the contemporaneous Society of Obstetricians and Gynaecologists of Canada criteria, including hypertension (blood pressure $\geq$140/90 mmHg, on at least two occasions >15 min apart after 20 weeks' gestation) with new proteinuria ($\geq$0.3 g/day by 24 h urine collection, $\geq$30 mg/mmol by protein:creatinine ratio, or $\geq$1+ by urinary dipstick) or one or more adverse conditions (e.g., headache/visual symptoms, chest pain/dyspnea, nausea or vomiting, right upper quadrant pain, elevated WBC count) or one or more severe complications (e.g., eclampsia, uncontrolled severe hypertension, platelet count <50×10^9/L, acute kidney injury) [31]. Women with chronic hypertension, known CVD prior to pregnancy, known kidney disease prior to pregnancy, or diabetes (type I, type II, gestational) were excluded. Small-for-gestational age (SGA) status was used as a proxy for fetal growth restriction and was defined conservatively as infant birth weight <5th percentile for gestational age at delivery and sex [32]. Clinical data, including medical and family history, pregnancy outcome, and postpartum cardiovascular health results, were collected by chart review following 6 months postpartum cardiovascular screening.

2.4. Placenta Pathology

For study participants in Ottawa, placentas were collected at the time of delivery and sent to the Pathology department. Trimmed placental weight was recorded, and gross pathology was recorded by an experienced pathology assistant. Four full-thickness tissue biopsies were randomly excised from each quadrant of the placenta, between the cord insertion site and the placental margins. Areas of visible pathology were sampled separately and not used for the full-thickness sections. Tissue was fixed in 4% neutral buffered formalin and paraffin-embedded. Following sectioning (5 µm), the tissue was stained with hematoxylin and eosin (H&E) using standard protocol [33] and stored for examination. In Kingston, archived H&E-stained tissue slides (4–5 slides/participant) were accessed from the Pathology archives for each participant. Sampling procedures were similar to those followed in Ottawa in that full-thickness biopsies were excised from each quadrant of the placenta, between the margin and cord insertion site.

At both study sites, trimmed placenta weight and gross pathology were collected from the accompanying placental pathology reports. A single, experienced placental pathologist examined the stained slides from all study participants, blinded to all clinical information apart from gestational age at delivery. Placental lesions were evaluated according to a standardized placental pathology data collection form, with pre-specified severity criteria derived from clinical practice guidelines and published literature [34]. Within the evaluation scheme, each lesion has a severity score to achieve a quantitative output for the severity of pathology. Lesions were either given a binary score of 0 (absent) or 1 (present) or a graded score according to a linear scale (i.e., 0 = absent, 1 = focal, 2 = patchy, 3 = diffuse). Individual lesions are grouped according to broad etiological categories, with a maximum severity score calculated for each category. Lesion categories and maximum severity score for each category are as follows: MVM (max score 14), implantation site abnormalities (max score 4), histological chorioamnionitis (max score 11), placental villous maldevelopment (max score 5), fetal vascular malperfusion (max score 6), maternal-fetal interface disturbance (max score 5), and chronic inflammation (max score 6). Gross anatomy (e.g., placental weight, umbilical cord length) was obtained from the corresponding historical placental pathology reports, in addition to several microscopic lesions (e.g., placental infarction, chronic deciduitis), as the tissue biopsies were collected from areas that appeared grossly normal and only included villous parenchyma (i.e., maternal decidua was not sampled).

2.5. Cardiovascular Risk Assessment

At 6 months postpartum, all women underwent physical and biochemical CVR screening in the Maternal Health Clinic (Kingston General Hospital) or at the Ottawa Hospital (Ottawa) according to published protocol [29]. A full medical history was taken and included information on family history, breastfeeding, and lifestyle such as smoking status and alcohol intake. A physical examination, specifically focusing on cardiovascular-related clinical predictors, was performed and collected information on weight, height, and blood pressure. A maternal blood sample was collected, and total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, glucose, and high sensitivity C-reactive protein were quantified for each participant. Physical and biochemical CVR findings were integrated to calculate a lifetime risk score for CVD, according to the previously published protocol [29]. Calculations for lifetime cardiovascular risk are based on the following risk factors: total cholesterol, systolic blood pressure, diastolic blood pressure, use of anti-hypertensive medication(s), fasting blood glucose, diagnosis of diabetes, and current smoking status. Risk stratification for each risk factor was based on predetermined measurement thresholds (optimal, not optimal, elevated, major). Lifetime cardiovascular risk estimates are also categorical and based on the total number of optimal, not optimal, elevated, and major risk factors of each individual (8%, all risk factors are optimal; 27%, $\geq$1 risk factor is not optimal; 39%, $\geq$1 risk factor is elevated; 39%, 1 risk factor is major; 50%, $\geq$2 risk factors are major). Lifetime cardiovascular risk estimates were simplified to categorize the women as low risk (<39% risk) or high risk ($\geq$39% risk) for lifetime CVD. This threshold corresponds to the baseline lifetime CVD risk attributed to healthy women enrolled in the Framingham Heart Study [35].

2.6. Statistical Analysis

Data were analyzed using SPSS 26.0 (SPSS Inc., Chicago, IL, USA). Descriptive data were expressed as means and standard deviations for normally distributed data or medians with interquartile ranges for non-normally distributed data. Chi-square tests were used for the comparison of categorical variables, while Student's *t*-test or Mann–Whitney U-tests were used for continuous variables. Placental lesions (frequencies and severity scores) were compared between the low CVD risk and high CVD risk groups. The association between individual placental lesions and lifetime CVD risk was assessed using odds ratios (OR) with 95% confidence intervals. Multivariable logistic regression was used to develop prediction models for lifetime CVD risk with placental data alone or in combination with clinical data. The performance of the models was assessed using area under the receiver operator characteristic (AUC ROC) curve analysis. Statistical significance was defined as $p < 0.05$.

3. Results

3.1. Clinical Characteristics

A total of 85 women were included in this study (35 from Ottawa and 50 from Kingston). The clinical characteristics of the participants, as a combined cohort and by each study site, are shown in Tables 1 and 2. The demographic characteristics of the women between the two study sites were not significantly different, apart from maternal age and pre-pregnancy BMI. Although the average age of women in Kingston was 2 years younger than the women in Ottawa ($p = 0.015$), the mean age of participants at both sites was <34 years (31.9 $\pm$ 6.0 vs. 33.9 $\pm$ 5.6), and this was not deemed to be of high clinical relevance. Although women in Ottawa had significantly higher pre-pregnancy BMIs than women in Kingston ($p = 0.024$), the gestational weight gain for the index pregnancy was similar between the two sites (13.2 $\pm$ 7.1 vs. 14.6 $\pm$ 7.1, $p = 0.393$).

Table 1. Maternal characteristics of the study participants as a combined cohort and by individual study site.

	Combined (n = 85)	Ottawa (n = 35)	Kingston (n = 50)	p-Value [§] (t-Test, KW, X^2)
Maternal Characteristics				
Maternal age at delivery (y)	31.9 ± 6.0	33.9 ± 5.6	30.7 ± 5.9	**0.015**
Postsecondary education (%)	74 (88.1) [a]	31 (91.2) [a]	43 (86.0)	0.520
Married or common law	80 (95.2) [a]	35 (100)	45 (91.8) [a]	0.137
Nulliparous (%)	59 (69.4)	22 (62.9)	37 (74.0)	0.341
Pre-pregnancy BMI	24.5 (22.1, 31.0)	28.2 (23.0, 35.5)	24.4 (21.9, 28.5)	**0.024**
Smoking (%)	6 (7.1)	1 (2.9)	5 (10.0)	0.393
Previous history of HDPs (%)	11 (12.9)	7 (20.0)	4 (8.0)	0.187
Family history of CVD * (%)	44 (52.4) [a]	16 (47.1) [a]	28 (56.0)	0.506
Family history of PE (%)	12 (15.0) (n = 80)	6 (20.0) (n = 30)	6 (12.0)	0.520

Data presented as mean ± SD, median (IQR) or n (%). BMI: body mass index; CVD: cardiovascular disease; HDP: hypertensive disorders of pregnancy; IQR: interquartile range; PE: preeclampsia; SD: standard deviation. [§] Comparison between participants in Ottawa and Kingston. * Includes coronary artery disease and cerebral vascular disease. [a] Data missing for one participant.

Table 2. Delivery and postpartum characteristics of the study participants as a combined cohort and by individual study site.

	Combined (n = 85)	Ottawa (n = 35)	Kingston (n = 50)	p-Value [§] (t-Test, KW, X^2)
At delivery				
Systolic BP *(mmHg)	152 ± 25	136 ± 17	164 ± 22	**<0.0001**
Diastolic BP *(mmHg)	93 ± 13	85 ± 10	98 ± 13	**<0.0001**
Antihypertensive medication ** (%)	38 (44.7)	28 (80.0)	6 (12.0)	**<0.0001**
Pregnancy weight gain (kg)	14.0 ± 7.1	13.2 ± 7.1	14.6 ± 7.1	0.393
Gestational age delivery	36.0 (32.2, 38.0)	37.5 (34.4, 39.4)	34.0 (31.0, 38.0)	**<0.001**
Delivery before 37 weeks gestation (%)	48 (56.5)	11 (31.4)	37 (74.0)	**<0.001**
Cesarean section (%)	44 (51.8)	14 (40.0)	30 (60.0)	0.081
Female infant (%)	42 (49.4)	13 (37.1)	29 (58.0)	0.078
Birth weight (g)	2200 (1495, 3098)	2655 (2075, 3280)	1920 (1285, 2351)	**0.0003**
Small for gestational age (<5th percentile)	15 (17.6)	5 (14.3)	10 (20.0)	0.573
Admission to NICU (%)	59 (69.4)	15 (42.9)	44 (88.0)	**<0.001**
Placental weight (g)	334 (274, 443)	382 (326, 516)	312 (236, 431)	0.057
At 6 months postpartum				
Systolic BP (mmHg)	119 ± 18	116 ± 23	121 ± 13	0.164
Diastolic BP (mmHg)	81 ± 10	78 ± 9	82 ± 10	0.081
Antihypertensive medication use (%)	13 (15.3)	5 (14.3)	8 (16.0)	1.00
Breastfeeding (%)	44 (52.4)	22 (64.7)	22 (44.0)	0.077
Total cholesterol	4.8 ± 1.0	4.9 ± 1.0	4.7 ± 1.0	0.292
Fasting glucose	4.8 ± 0.5	4.7 ± 0.5	4.8 ± 0.5	0.397
HDL	1.5 ± 0.4	1.5 ± 0.4	1.5 ± 0.4	0.541
LDL	2.8 (2.2, 3.4)	3.0 (2.2, 3.5)	2.6 (2.1, 3.3)	0.231
hsCRP	2.6 (1.0, 7.4)	2.6 (0.9, 8.4)	2.0 (0.98, 5.9)	0.443
Triglycerides	0.98 (0.67, 1.69)	0.98 (0.72, 1.88)	0.96 (0.65, 1.60)	0.500
Screen high-risk for lifetime CVD (%)	53 (62.4)	18 (51.4)	35 (70.0)	0.112

Data presented as mean ± SD, median (IQR) or n (%). BP: blood pressure; hsCRP: high sensitivity C-reactive protein; CVD: cardiovascular disease; HDL: high density lipoprotein; IQR: interquartile range; LDL: low density lipoprotein; NICU: neonatal intensive care unit; PE: preeclampsia; SD: standard deviation. [§] Comparison between participants in Ottawa and Kingston. * Highest BP measurement following admission before delivery. ** Medication started during index pregnancy or postpartum prior to discharge from hospital.

As for pregnancy outcomes, women in Kingston had significantly higher blood pressures at delivery, increased use of anti-hypertensive medication in pregnancy, and delivered at earlier gestational ages compared to women in Ottawa (34.0 (31.0, 38.0) weeks vs. 37.5 (34.4, 39.4), $p < 0.0001$) and had more SGA infants compared with women in Ottawa. At 6 months postpartum, there were no significant differences in cardiometabolic parameters between the participants at the study sites. Of the 85 women included in the analysis, 53 (62.4%) women screened high-risk for lifetime CVD at 6 months postpartum.

3.2. Histopathology Findings in Low- and High-Risk Women

The frequency of placenta lesions between women who screened as low- and high-risk for lifetime CVD are shown in Table 3. A total of 5 placentas (15.6%) in the low-risk group and 6 placentas (11.3%) in the high-risk group had no observed pathology ($p = 0.74$). The mean cumulative severity score (sum of scores for all categories) for the low-risk group was 3.1 $\pm$ 2.2 and 3.6 $\pm$ 2.4 for the high-risk group ($p = 0.374$). By etiological category, women who screened as high-risk for lifetime CVD had placentas with more severe lesions of MVM (score $\geq$ 2: 54.7% vs. 28.1%, $p = 0.017$); however, the frequency of individual lesions belonging to the MVM category was not found to be significantly different between the groups. There were no differences in the frequencies of placental lesions between high and low-risk groups when stratified by individual study site (data not shown).

Table 3. Frequency of placental lesions by cardiovascular risk group.

Placental Lesion	High CVD Risk ($n = 53$)	Low CVD Risk ($n = 32$)	p-Value (Pearson X^2)
Evidence of maternal vascular malperfusion			
Placental infarction	16 (30.2)	7 (21.9)	0.403
Distal villous hypoplasia	15 (28.3)	8 (25.0)	0.740
Accelerated villous maturation	31 (58.5)	12 (37.5)	0.061
Syncytial knots	34 (64.2)	17 (53.1)	0.315
Perivillous fibrin deposition	5 (9.4)	6 (18.8)	0.215
Villous agglutination	7 (13.2)	1 (3.1)	0.123
Presence of retroplacental hematoma	0 (0)	2 (6.3)	0.066
MVM Score of 0	8 (15.1)	9 (28.1)	0.146
MVM Score 2 or more	29 (54.7)	9 (28.1)	**0.017**
Evidence of maternal decidual arteriopathy			
Insufficient vessel remodeling	7 (13.2)	2 (6.3)	0.312
Fibrinoid necrosis	4 (7.5)	2 (6.3)	0.821
Decidual arteriopathy present	9 (17.0)	3 (10.3)	0.416
Evidence of ascending intrauterine infection			
Maternal inflammatory response	2 (3.8)	4 (4.7)	0.128
Fetal inflammatory response	2 (3.8)	2 (6.3)	0.601
Ascending intrauterine infection present	3 (5.7)	5 (15.6)	0.127
Evidence of placenta villous maldevelopment			
Chorangiosis	0 (0)	0 (0)	–
Chorangiomas	0 (0)	0 (0)	–
Delayed villous maturation	1 (1.9)	2 (6.3)	0.291
Evidence of fetal vascular malperfusion			
Avascular fibrotic villi	2 (3.8)	0 (0)	0.266
Thrombosis	1 (1.9)	1 (3.1)	0.715
Intramural fibrin deposition	0 (0)	3 (9.4)	**0.023**
Karyorrhexis	0 (0)	0 (0)	–
High-grade fetal vascular malperfusion	2 (3.8)	0 (0)	0.266
Fetal vascular malperfusion present	4 (7.5)	5 (15.6)	0.241

Table 3. *Cont.*

Placental Lesion	High CVD Risk (n = 53)	Low CVD Risk (n = 32)	p-Value (Pearson X^2)
Fibrinoid			
Massive Perivillous fibrin deposition pattern	1 (1.9)	0 (0)	0.434
Maternal floor infarction pattern	0 (0)	0 (0)	–
Intervillous thrombi			
Intervillous thrombi	5 (9.4)	1 (3.1)	0.271
Evidence of chronic inflammation			
Villitis of unknown etiology	5 (9.4)	3 (9.4)	0.993
Chronic intervillositis	0 (0)	0 (0)	–
Chronic plasma cell deciduitis	5 (9.4)	3 (9.4)	0.993
Chronic inflammation present	7 (13.2)	6 (18.8)	0.492

Data presented as *n* (%). MVM: maternal vascular malperfusion.

3.3. Association of Placental Lesions and CVD Risk

Individually, no placental lesions were found to be significantly associated with high-risk CVD screening at 6 months postpartum. However, PE women with lesions of MVM with a severity score of 2 or more were more likely to screen high-risk for lifetime CVD than PE women without severe MVM lesions (severity score <2) (OR 3.10 (1.20–7.92)). Clinical data in the absence of placenta pathology findings (maternal age, gestational weight gain, blood pressure at delivery, gestational age at delivery) was moderately predictive of high-risk screening at 6 months postpartum AUC 0.68 (0.55, 0.81); sensitivity: 86.5% (74.7, 93.3); specificity: 29.0% (16.1, 46.6)) (Figure 1a). Severity of MVM lesions alone (score 2 or more) was similarly predictive of high-risk screening at 6 months postpartum (AUC 0.63 (0.51, 0.75); sensitivity: 71.8% (54.6, 84.4); specificity: 54.7% (41.5, 67.3)) (Figure 1b). However, when clinical data and MVM lesion severity were combined, the model's predictive performance improved (AUC 0.73 (0.62, 0.84) sensitivity 78.4% (65.4, 87.5); specificity 51.6% (34.8, 68.0)) (Figure 1c).

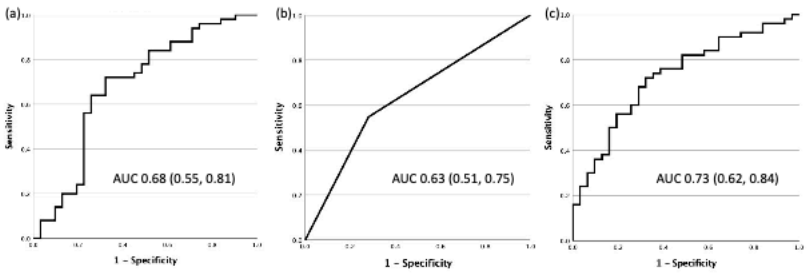

Figure 1. Area under the receiver operator characteristic curves for the prediction of screening high-risk for lifetime cardiovascular disease at 6 months postpartum by (**a**) maternal age, gestational weight gain, blood pressure at delivery, and gestational age at delivery, (**b**) maternal vascular malperfusion severity score, and (**c**) maternal vascular malperfusion severity score, maternal age, gestational weight gain, blood pressure at delivery, and gestational age at delivery.

4. Discussion

4.1. Main Findings

In this study, we observed an increase in placental histopathological lesions in women who screened high risk for lifetime CVD at 6 months postpartum following a PE pregnancy. Specifically, high-risk women had more severe lesions of MVM, the placental pathology traditionally associated with PE. MVM lesions with a severity score >2 resulted in a 3-fold increased risk of screening high risk for lifetime CVD at 6 months postpartum. The cumulative severity of MVM lesions was important in this association, suggesting that there may be critical thresholds of placental damage that reflect increased lifetime cardiovascular risk.

4.2. Interpretation

Previous studies have demonstrated an association between placental pathology and increased postpartum maternal cardiovascular health risk [21–26]. One study found that in normotensive women who experienced placental abruption during pregnancy, CVRs were significantly altered 6–9 months postpartum compared to women without uncomplicated pregnancies [25]. Lesions of maternal vascular maldevelopment (defined as mural hyperplasia, unaltered decidual vessels, and decidual atherosis) are associated with maternal hypertension 7 to 15 years after pregnancy [36]. Catov et al. reported that in normotensive women who delivered preterm without fetal growth restriction, those who had placental lesions of MVM and inflammation had significantly elevated atherogenic profiles assessed 4–12 years after delivery [21]. Our findings are in line with this study in which the cumulative severity of placental lesions may be important for identifying women at the highest cardiovascular risk following pregnancy. Together with our current findings, significant placental pathology may be indicative of a greater risk for CVD postpartum.

The mechanisms linking PE and postpartum maternal cardiovascular risk have yet to be fully elucidated. The most commonly held hypothesis to explain this link proposes that pre-pregnancy maternal CVRs, including both clinically diagnosed and subclinical risk factors, may contribute to the development of PE, including abnormal placental development, and predispose women to CVD after pregnancy. Placentation requires the invasion of fetal trophoblast cells into the maternal decidua, resulting in the conversion of the maternal uterine spiral arteries to high capacity, venous-like conduits to increase blood flow into the uteroplacental unit to support fetal growth and development [37]. This physiological remodeling of the uterine spiral arteries is known to be defective in PE, and the two-stage model of pathogenesis proposes that this failed remodeling leads to placental ischemia, oxidative stress, and placental dysfunction, which stimulates the release of angiogenic factors, pro-inflammatory cytokines, syncytiotrophoblast vesicles, and other factors from the placenta [1]. These processes result in characteristic histopathological lesions observed in placentas from pregnancies complicated by PE, particularly lesions of MVM [38]. Placenta-derived circulating factors interact with the maternal endothelium at the systemic level, leading to the end-organ dysfunction observed in the clinical manifestation of the disorder. The maternal environment, including subclinical CVRs common to PE and CVD, may directly contribute to impaired trophoblast invasion and defective spiral artery conversion and its downstream effects. Dyslipidemia, including elevated pre-pregnancy levels of serum triglycerides, cholesterol, LDL, and non-HDL cholesterol, have been associated with increased risk of developing PE and are known contributors to CVD [39]. Studies have shown that lipids modulate human trophoblast invasion, and alterations in maternal lipid profiles could potentially contribute to abnormal trophoblast invasion and spiral artery remodeling [40,41]. Systemic (often subclinical) inflammation, common in obesity and other cardiometabolic conditions, may also play a role in limited trophoblast invasion and spiral artery conversion during placentation in PE [42,43]. Pro-inflammatory cytokines are known to inhibit trophoblast invasion by increasing apoptosis and decreasing proliferation [44]. Cytokine imbalance prior to pregnancy may alter the maternal inflammatory milieu over and above the physiological immune/inflammatory

changes that occur in pregnancy; however, exactly how this imbalance contributes to altered placentation is unknown.

PE and placental dysfunction, reflected as MVM lesions in the placenta, may also cause lasting damage to the maternal cardiovascular system that results in altered cardiovascular health trajectories. Circulating levels of inflammatory cytokines such as tumor necrosis factor-a (TNF-a) and interleukin-6 (IL-6) are elevated in PE and interfere with the maternal endothelium to produce systemic endothelial dysfunction. Women diagnosed with PE have been found to have chronically altered circulating levels of these cytokines >20 years after pregnancy, suggesting that PE may program the maternal cardiovasculature such that there is persistent cardiovascular dysregulation, precipitating CVD in later life [45]. Other maternal cardiometabolic pathways, including the renin–angiotensin system, may also contribute to abnormal placentation and reduced uteroplacental exchange, the two main features of PE [46]. While yet to be fully elucidated, dysregulation of these cardioregulatory pathways in the mother after pregnancy may contribute to the increased risk for CVD after PE [47]. Additionally, anti-angiogenic imbalance in the maternal circulation, including elevated soluble Fms-like tyrosine kinase-1 (sFlt-1) and reduced placental growth factor (PlGF), may contribute to lasting cardiovascular dysfunction after PE. Alternations in circulating angiogenic factors during pregnancy are associated with cardiovascular changes, including increased blood pressure 6 to 12 years after pregnancy [48,49]. Although angiogenic factor levels significantly drop following delivery, a recent study suggests that angiogenic imbalance may be persistent in the postpartum period [50]. While the mechanisms of angiogenic factors on cardiovascular health are not fully known, sFlt-1 and PlGF have been shown to influence vasodilatory function in preclinical models [51,52].

It is plausible that a combination of the pre-pregnancy maternal environment and persistent alterations to the maternal cardiovascular system from placental dysfunction contributes to future CVD risk. Placental pathology, particularly lesions of MVM, may reflect both abnormalities in the maternal milieu as well as the significant cardiovascular burden from abnormal placentation, thereby identifying patients at particularly increased risk of postpartum CVD. As such, placental lesions identified at the time of delivery could provide a modality to triage PE women for cardiovascular health screening postpartum. Future studies are required to confirm the utility of placental pathology in this capacity; it may offer a unique opportunity to extend the clinical benefits of the placenta pathology exam while targeting postpartum resource-intensive cardiovascular screening to the most vulnerable patients.

4.3. Strengths and Limitations

The limitations of our study need to be considered. First, we did observe a significant difference in several important pregnancy parameters between our study sites, including blood pressure at delivery, anti-hypertensive use at delivery, and gestational age at delivery—indicative of a more severe form of maternal disease in our Kingston cohort. We believe these differences are likely the result of sampling bias introduced by the different study designs used across the two sites. In Kingston, a retrospective analysis was performed on placentas originally submitted to pathology based on the clinical judgment of the treating physician. Despite a PE diagnosis being identified as an indicator for placenta pathology submission, only 53% of placentas were submitted for evaluation at this site during the study period (unpublished internal audit data), in line with previous literature on the topic of suboptimal placenta pathology submission practices [53]. On the other hand, a prospective study design was employed at the Ottawa site, with 100% placental pathology submission for recruited PE study participants, irrespective of the severity of their clinical characteristics. As cardiovascular parameters were similar between our cohorts at the 6-month postpartum clinic visit, we do not feel these delivery parameters influenced our findings. Due to our small sample size, we may be underpowered to detect differences between low and high-risk groups for less common pathology, such as chronic inflammation. However, for MVM lesions, we found that a sample size of 48 was needed

to detect differences between MVM scores greater than 2 within the high- and low-risk groups at the confidence level of 95% and power of 80%. Confirming our results in an adequately powered prospective study may identify a predictive combination of placental lesions that robustly identifies high-priority women for postpartum CVD screening at the time of delivery. Our study is strengthened by the standardized placental evaluations we conducted using our evidence-based synoptic data collection [34]. Variability in placenta pathology examination is a known challenge, and the use of this synoptic collection form ensures each placenta was evaluated in a reproducible manner. Moreover, our cohort study design reflects routine clinical practice in which only placentas from complicated pregnancies are submitted for histopathological examination. While placentas from uncomplicated pregnancies exhibit placental lesions with varying degrees of prevalence and severity [54], these placentas are not routinely sent for examination and the use of placental pathology in this population is limited.

5. Conclusions

Women with PE and severe lesions of MVM are more likely to screen as high-risk for lifetime CVD at 6 months postpartum compared to women without these lesions. Placenta pathology may provide a unique modality to identify women for postpartum cardiovascular screening. Triaging at the time of delivery would allow for targeted screening and resource allocation to the truly at-risk PE women.

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Chapter 4

Placental protein expression of fms-like tyrosine kinase-1 (FLT-1), endoglin (ENG), and cluster of differentiation 68 (CD68) and future maternal cardiovascular risk profiles following preeclampsia

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Abstract

Background: Preeclampsia (PE), a hypertensive disorder of pregnancy, is associated with an increased risk for future maternal cardiovascular disease (CVD). Previous placental gene expression profiling revealed 3 clinically distinct PE disease subclasses. These subclasses differ in severity of maternal disease, fetal growth patterns, placental gene expression, and placental histopathology. Notably, "Hypoxic PE" is associated with an increase in placental expression of FLT-1 and ENG. "Inflammatory PE" is associated with an increase in CD68+ immune cell infiltration into the placenta. "Maternal Maladaptation PE" is characterized by a lack of overt placental disease. Here, we explore if subclass-specific protein biomarkers of PE in the placenta can be used to identify women who demonstrated high lifetime cardiovascular risk profiles at 6 months postpartum.

Methods: Immunohistochemistry (IHC) analysis for FLT-1, ENG, and CD68 was performed on placental tissue biopsies from 35 women with PE and 6 normotensive controls. These participants had placental histopathology specimens collected at the time of delivery and underwent a cardiovascular risk assessment at 6 months postpartum. Image J was used to quantify FLT-1 and ENG expression within the syncytiotrophoblast layer of placental villi via the IHC profiler plugin. QuPath was used to quantify CD68-positive cells per tissue area. All IHC analysis was done blinded to pregnancy outcome and CVD risk profile. The association between PE subclass, proxied by FLT-1, ENG, and CD68 expression, and elevated lifetime cardiovascular risk was assessed by multivariable logistic regression and receiver operator curve analysis.

Results: Individually, no significant differences were found in FLT-1, ENG, or CD68 expression between the high and low-risk groups. Although, when expression for all three markers was

combined in a logistic regression model, moderate prediction of elevated risk for CVD was observed (AUC: 0.64 [0.46-0.82]). When added to a model with clinical characteristics and placental pathology findings, the performance increased to AUC: 1.00 [1.0-1.0].

Conclusions: In conclusion, individual differences in FLT-1, ENG, and CD68 expression by cardiovascular risk status were not observed when measured by IHC. However, they should continue to be explored in a larger sample and possibly using different methods of quantification, as their implementation into clinical models improved prediction of elevated risk for CVD to 100%. These results demonstrate further evidence of the utility of unique placenta features in the prediction of lifetime CVD risk status following a PE pregnancy.

Keywords: Placenta; Preeclampsia; Immunohistochemistry; Cardiovascular Disease; Cardiovascular Risk; Endoglin; CD68; FLT-1; Disease Subtypes: Hypoxia; Inflammation; Placental Macrophages; DAB staining

1. Introduction

Preeclampsia (PE) is a hypertensive disorder occurring within 3-8% of all pregnancies [1]. It is defined by de novo hypertension after 20 weeks' gestation and maternal end-organ dysfunction [2]. Globally, it is one of the leading causes of maternal, fetal, and neonatal mortality, responsible for 60,000 maternal and 500,000 perinatal deaths per year [3]. The consequences of PE can extend beyond the peripartum period, with life-long health implications for both mother and child [4,5]. Importantly, PE is an independent risk factor for future maternal cardiovascular disease (CVD) [4]. It is associated with a 2.5-fold increase in cardiovascular mortality, 3.7-fold increase for chronic hypertension, and 4.19-fold increase for heart failure [6]. To reduce the burden of future CVD, postpartum cardiovascular risk assessments are recommended following PE [7].

Although the pathogenesis of PE is yet to be completely elucidated, in most instances it appears to be placenta-mediated [1]. According to the well described disease paradigm, it is initiated by insufficient placental invasion of the uterine wall and spiral artery remodelling [1]. This leads to placental hypoperfusion and hypoxia triggering the release of placental stress-induced factors into maternal circulation [1]. Soluble fms-like tyrosine kinase receptor-1 (sFLT-1) and soluble endoglin (sENG) are two anti-angiogenic factors produced by the placenta and released into the maternal circulation at increased levels in PE pregnancies [8-10]. sFLT-1 is an inhibitor of vascular endothelial growth factor (VEGF) and placental growth factor (PIGF), preventing their angiogenic activity on vascular tissues [8]. sENG modulates the activity of transforming growth factor beta (TGFB) isoforms [10]. As such, it can impair downstream activation of endothelial nitric oxidase synthase and subsequent vasodilation of endothelial

tissues [10]. Together, increased placental secretion of sFLT-1 and sENG can lead to systemic maternal endothelial dysfunction causing the systemic hypertension and end-organ damage observed in PE cases [10,11]. The involvement of placental sFLT-1 and sENG in PE is further supported by their increased presence in the maternal circulation before onset of clinical symptoms [12,13], increase along with disease severity [9,10,14], decrease after delivery [9,10,14], and the induction of PE symptoms in animal models with sFLT-1 or sENG overexpression [10,11]. Membrane bound variants of FLT-1 and ENG are also expressed by vascular endothelial cells and placental trophoblasts, although the soluble variants appear to be primarily upregulated in instances of PE [15,16]. Despite the support for sFLT-1 and sENG involvement in PE pathogenesis, not all women with PE demonstrate an elevation of these anti-angiogenic factors, indicating that PE may be a heterogenous disorder with several different etiologies [17,18].

Other possible etiologies include immune, genetic, and environmental factors [17-19]. Of note, maternal-fetal allograft rejection may initiate an inflammatory response and contribute to PE pathogenesis [17,18,20]. This is supported by an increased number of monocytes and activated M1 macrophages in PE pregnancies [21]. Although these cells are essential to trophoblast invasion and decidual formation in normal pregnancies, their excessive presence can result in placental malformations as observed in PE cases [20]. In addition, placental hypoxia in PE can also trigger the release of pro-inflammatory cytokines such as tumor necrosis factor alpha and interleukin 6 [20]. Collectively, these factors can contribute to a systemic maternal inflammatory response and further contribute to abnormal placentation and maternal endothelial dysfunction described in the previous two-stage disease model [1,20].

Research by Leavy et al. further explores the multifactorial nature of PE via unsupervised multivariate clustering of placental microarray and histopathology data [17,18]. Their results demonstrated 3 clinically distinct molecular subclasses of PE with divergent etiologies [17]. “Hypoxic PE”, the largest subclass of patients identified, demonstrated the most severe forms of maternal disease, earlier deliveries, lower placental weights, placental histopathology consistent with maternal vascular malperfusion (MVM), and increased expression of hypoxic markers including FLT-1, ENG, leptin (LEP) and inhibin subunit beta A (INHBA) [17,18]. The second subclass, coined “Inflammatory PE”, demonstrated evidence of a pro-inflammatory response at the maternal-fetal interface, with increased expression of chemokine C-X-C motif ligand 10 (CXCL10), a rejection marker, and ATP-binding cassette subfamily B (TAP1), an inflammatory marker [14]. This subclass was clinically characterized by late pre-term deliveries (34-37 weeks), lower birthweights and increased likelihood of fetal growth restriction, and placental histopathology consistent with maternal-fetal allograft rejection [17,18]. A more recent exploratory immunohistochemistry study of this PE subclass found an increase in placental infiltration with CD68+ cells (monocytes and macrophages) as well as myeloperoxidase+ cells (neutrophils), when compared to the Hypoxic PE subclass [22]. A third and smaller subclass, “Maternal-Maladaptation PE”, demonstrated minimal evidence of placental histopathology, normal placental gene expression profiles, appropriate for gestational age infants and normal placental weights despite a PE diagnosis [17,18] – findings indicative of a maternal predisposition to disease rather than a placental origin.

Recent studies have demonstrated evidence for the potential value of clinical placenta pathology evaluation in predicting future maternal health outcomes [23]. Notability, preliminary

studies demonstrate an association between placental MVM lesions and altered cardiometabolic profiles in the immediate postpartum period as well as 8-10 years later [23-25]. As these lesions are a hallmark feature of the hypoxic PE subclass, it is postulated that this specific group of PE women may be at highest risk for future CVD [25]. Here we aim to extend these findings by examining the association between placental immuno-expression of FLT-1, ENG, and CD68 – protein biomarkers of the hypoxic and inflammatory subclasses of PE respectively - and postpartum lifetime cardiovascular risk estimates to further understand the CVD risk profiles associated with each PE subclass. The findings of the current study may demonstrate that a simple IHC screening panel carried out at the time of delivery may prove useful in the development of effective triage strategies for referrals to specialized postpartum cardiovascular screening clinics following a pregnancy complicated by PE [26].

2. Materials and Methods

Study Recruitment

58 participants were initially recruited for the Determining the Risk Elevation after Maternity (DREAM) cohort study, from inpatient services at The Ottawa General Hospital (Ottawa, ON, Canada) between 2016 and 2018. The primary aims of this pilot prospective cohort study were to identify promising immune and anti-angiogenic biomarkers that may be predictive of future maternal cardiovascular risk. Those included in the cohort had a diagnosis of PE prior to delivery, maternal age between 18-45 years, and a singleton pregnancy. PE was defined in accordance with the current Society of Obstetrics and Gynecologists of Canada definition [2]. Participants with a history of chronic hypertension, CVD, kidney disease, or diabetes (type I, type II, or gestational) were excluded from the study. In addition, 10 participants with normotensive

pregnancies were recruited from the same hospital site as experimental controls. All participants provided written informed consent prior to study commencement. Basic clinical information and placental histopathological findings were obtained from a previous study with these participants [25; Chapter 3/Manuscript 2].

Cardiovascular Risk Assessment

At six months postpartum, participants completed a CVD risk assessment at the Ottawa General Hospital by a research study nurse. This assessment used clinical and biochemical risk factors to calculate a life-time CVD risk score validated for use in populations of women of reproductive age, as previously described [27]. Based on this score, participants were classified as high- or low-risk for lifetime CVD, where high-risk was defined as a risk score greater or equal to 39% [27]. A summary of the CVD risk screening tool is included in **Appendix A.2.** Although 58 participants were initially enrolled into the original DREAM study and had placenta samples available for IHC analysis, only 73% of PE participants (n=35) and 60% of normotensive controls (n=6) attended the scheduled follow-up appointment where CVD risk assessment screening was performed, and hence our specific analysis of associations between placental IHC markers and determined CVD risk estimates was limited to n=41 (**Figure 4.1**).

Immunohistochemistry

Five representative biopsies of the placental disk from all originally recruited participants (n=58) were sectioned, fixed, mounted, and wax-embedded following delivery. Using a standard immunohistochemistry (IHC) protocol, the relative staining of monoclonal anti-human FLT-1 (Abcam, USA; dilution 1:200), ENG (Sigma Aldrich, USA; dilution: 1:1000) and CD68 (Sigma Aldrich, USA; dilution: 1:1000) was measured in serial sections of each placental sample [28]. As

antibodies specific for the soluble variants of FLT-1 and ENG are still under development, generalized FLT-1 and ENG antibodies are used to detect both soluble and membrane bound variants [29,30]. As minimal changes in membrane bound FLT-1 and ENG are reported in PE, it is proposed that observed differences can be attributed primarily to the soluble variants [15,16]. Biotinylated anti-rabbit secondary antibodies were used according to manufacturer's instructions (DAKO LSAB kit, Agilent, USA; Dilution: 1:400) following DAB chromogen solution and DAB enhancer (Agilent, USA). All samples were counterstained with Hematoxylin. Full-section images of stained samples were captured with the Axio Scan Z1 Slide Scanner (Zeiss, Germany) at 20X magnification at the University of Ottawa (Ottawa, Canada).

Quantification

Four representative snapshots were taken at random at 20X magnification with Zeiss Zen Lite (Zeiss, Germany) for each FLT-1 and ENG-stained sample. Semi-quantification of FLT-1 and ENG was completed with the Image J (National Institutes of Health, USA) IHC profiler plugin, using a macro generated based on the quantification protocol of Crowe et al. to facilitate measurement of protein staining specifically within the syncytiotrophoblast layer of the villous tissue [31,32] (**Figure 4.2**) – the site of specific up-regulation in cases of PE. Briefly, a duplicate of the original image was obtained and converted to 8-bit form. A mask image, used to highlight a specific region of interest, was then created on this duplicate with the threshold function where the syncytiotrophoblast region of placental villi was selected. The threshold level was validated manually for each study image (**Figure 4.2**). If necessary, the threshold value was manually adjusted to ensure the syncytiotrophoblast cellular layer was captured within the mask. The range of lower limit threshold parameters used was 86-160 for FLT-1 and 80-159 for ENG stained

images (respective averages: 123 and 120). The IHC profiler plugin was used to obtain the DAB staining layer from the original image using colour deconvolution. The mask was then applied to the DAB layer and the mean grey value was measured within the region of interest (syncytiotrophoblast cellular layer) depicted in the mask. The mean grey value, representing average immunostaining intensity, was calculated for each snapshot, and the average of each 4 snapshot image set calculated for each original FLT-1 and ENG stained image. The mean grey values were compared between the PE and control groups (n=58) as well as between high and low CVD risk groups (n=41). The mean grey values ranged from 0-255 where 0 represented true white and 255 represented true black. The average mean grey value for PE samples was used as a threshold to assess elevations in FLT-1 and ENG expression by cardiovascular risk score.

As CD68 identifies immune cells present within placental villi, a different approach was used for its quantification. CD68 quantification within each full-slide image was computed using the positive cell detection feature set to DAB and Hematoxylin stained tissues from Qupath (University of Edinburgh, Scotland) **(Figure 4.3)**. Total villi tissue area was calculated using Image J, with the auto-threshold function. The number of CD68+ cells per villi tissue area was calculated for each full-section sample. The number of CD68+ cells per villi tissue area was compared between the PE and control groups (n=58) as well as between high and low CVD risk groups (n=41). The average number of CD68+ cells per villi for PE samples was used as a threshold to assess elevated CD68+ cells by cardiovascular risk score.

Statistical Analysis

SPSS 26.0 (SPSS Inc., USA) was used for data analysis. Data normality was tested by the Shapiro-Wilk Test for all continuous variables. Descriptive data is reported as medians and

interquartile range for non-normally distributed variables, means and standard deviation for normally distributed variables, and frequency and percentage for categorical variables. For comparison between the PE and control groups, Mann-Whitney U Test was used for non-normally distributed variables, Student's T-Test for normally distributed variables, and Pearson Chi-Squared for categorical variables. An additional comparison was performed by cardiovascular risk status with the appropriate statistical tests. Continuous FLT-1, ENG, and CD68 expression was compared by pregnancy outcome (PE vs Control) and lifetime cardiovascular risk score (High vs Low). Differences between groups were assessed by Mann-Whitney U Test. Multivariable logistic regression was used to develop predictive models for lifetime CVD risk with placental protein expression, placenta histopathology and clinical data. Model performance was assessed by receiver operator characteristic (ROC) and is expressed by area under the curve (AUC). Statistical significance was evaluated at $p<0.05$.

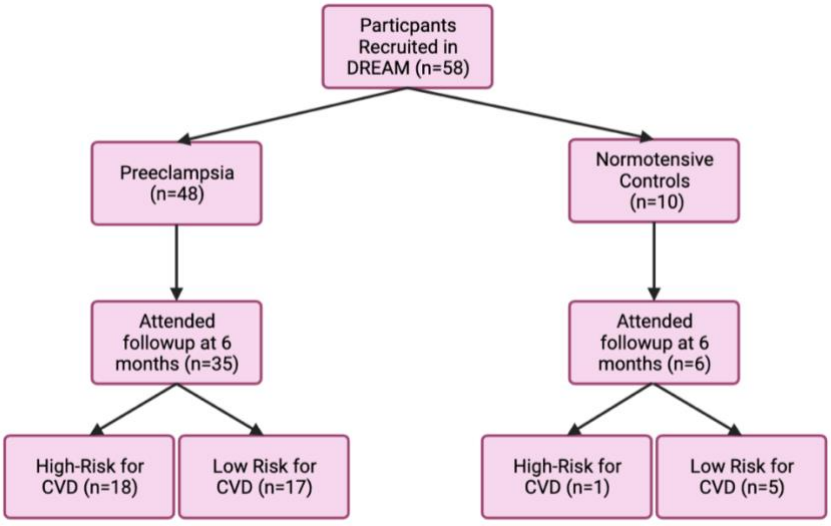


Figure 4.1 Study Group Organization. Initially, 58 participants were recruited into the DREAM study (48 PE and 10 normotensive controls). Of these, 35 PE and 6 controls attended the CVD risk screening assessment at 6 months postpartum (n=41). 18 PE women and 1 control screened high-risk for CVD. Created with Biorender.

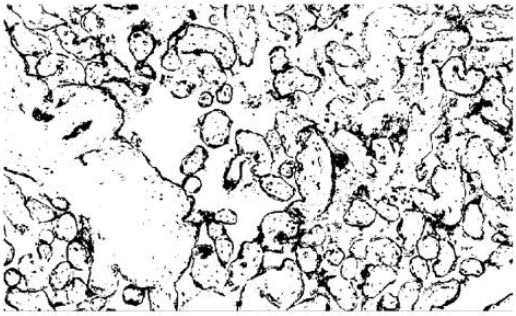
A.

```
// Open each image, duplicate it, and create a mask of the syncytiotrophoblast
rename("Sample");
run("Set Scale...", "distance=155 known=100 unit=um");
run("Duplicate...", "title=Mask");
run("8-bit");
run("Threshold...");  // open Threshold tool
title = "WaitForUserDemo";
msg = "If necessary, use the \"Threshold\" tool to adjust the threshold, then click \"OK\".";
waitForUser(title, msg);
getThreshold(lower, upper);
setOption("BlackBackground", false);
run("Convert to Mask");
run("Create Selection");

// Run the IHC profiler and obtain the DAB staining
selectWindow("Sample");
run("IHC Profiler", "mode=[Cytoplasmic Stained Image] vectors=[H DAB]");
close();
run("Set Scale...", "distance=155 known=100 unit=um");
rename("dabImage");

// Use the mask to determine the regions (syncytiotrophoblast) and select them on the DAB image
run("Restore Selection");
run("Set Measurements...", "area mean standard integrated limit display redirect=None decimal=3");
run("Measure");
showMessage("Done!")
selectWindow("Sample");
close("\\Others")
run("Open Next");
runMacro("One by One macro.txt");
```

B.



C.

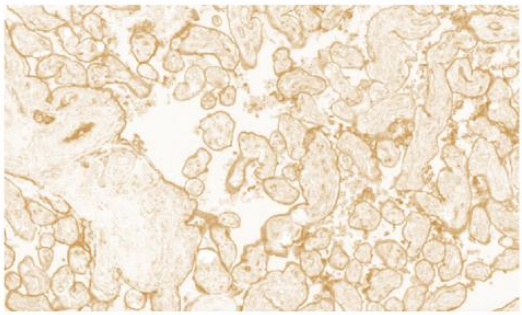


Figure 4.2 Macro for Semi-Quantification of FLT-1 and ENG Expression in Placental Syncytiotrophoblast. Full-slide digital images of participant placental specimens were captured at 20X magnification on Aperio Scanscope after immunohistochemistry DAB staining with anti-FLT-1 or anti-ENG antibodies, with mean immunostaining intensity (mean grey value) calculated within the syncytiotrophoblast region using the IHC profiler on Image J and an automated macro. **A.** Macro of semi-quantification protocol for FLT-1 and ENG immunostained images. Briefly, the threshold tool was used to select the syncytiotrophoblast region manually. This selection was converted to a mask and applied to the extracted DAB staining layer obtained with the IHC profiler. The area and mean grey value for this region were measured. **B.** Mask of syncytiotrophoblast layer obtained by thresholding for the region of interest in a sample snapshot ("Mask"). **C.** Immunopositive DAB staining layer obtained by colour deconvolution for the same sample snapshot ("dabImage").

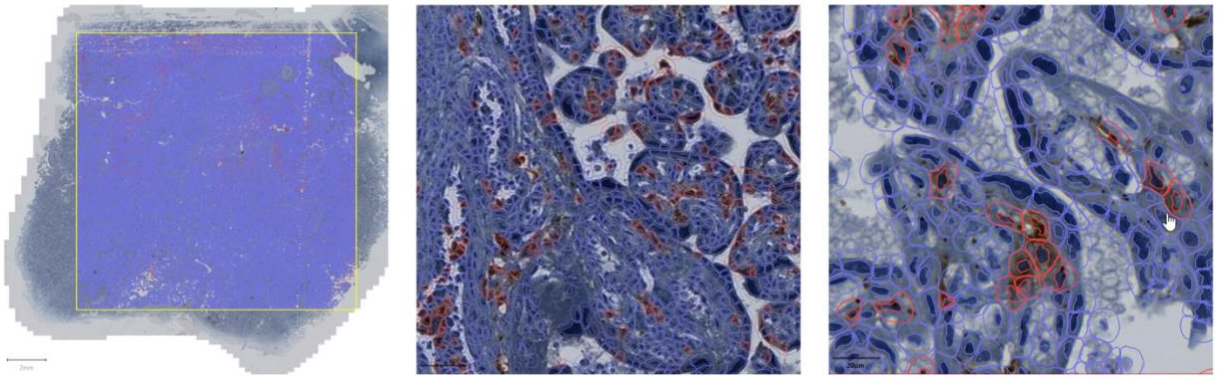


Figure 4.3 Positive Cell Detection of CD68 Expression in Placental Sections. Full-slide digital images of participant placental specimens were captured at 20X magnification on Aperio Scanscope after immunohistochemistry analysis with immunohistochemistry DAB staining with anti-CD68 antibody. CD68 immunopositive cell detection (DAB-positive) was performed in QuPath. The total number of positive cells per villi area was calculated for each sample. **A**. A full-slide image of a representative digital image captured at 20X. **B-C**. Higher magnification snapshots of the same digital image, at an additional 10% and 20% respectively, highlighting immunopositive cells (highlighted in red) and immunonegative cells (highlighted in blue).

3. Results

3.1 Clinical Characteristics

A total of 41 women were included in the analysis for lifetime cardiovascular risk (35 PE and 6 controls). Clinical characteristics by estimated lifetime cardiovascular risk score are shown in **Tables 4.1 and 4.2** and pregnancy outcome in **Tables S1 and S2** (Supplementary Data). Approximately half of all PE patients (18/35, 51.4%) screened high risk at 6 months postpartum – in line with previous reports [33]. Unsurprisingly, the large majority (18/19, 94.7%) of patients deemed at high risk for CVD had a PE diagnosis, however 1 control patient (5.3%) also screened high risk. No significant differences were observed between the high and low lifetime CVD risk groups for baseline demographics apart from history of placenta mediated disease (**Table 4.1**; p=0.053). In addition, no significant differences were observed in these parameters when stratified by pregnancy outcome, apart from nulliparity (**Table S1**; p=0.035).

With regards to delivery characteristics, women who screened high-risk for lifetime CVD had significantly earlier deliveries compared to those who screened low-risk (p=0.047 for gestational age at delivery, p=0.040 for preterm birth) (**Table 4.2**). More adverse fetal outcomes were observed in the high-risk group evidenced by increased admission to NICU (p=0.047) and decreased placental weight (0.037) (**Table 4.2**). When comparing these characteristics by pregnancy outcome, PE women had significantly higher blood pressures at delivery (p<0.001, p=0.003), increased use of anti-hypertensive medications in pregnancy (p<0.001), and earlier deliveries compared to controls (p=0.004) (**Table S1**). More adverse fetal outcomes were observed in the PE group evidenced by decreased birth weight (p<0.001), decreased birth weight percentiles (p=0.016), decreased placental weights (p=0.003), and increased admission to NICU (p=0.044) (**Table S1**). Although, no significant differences were found between placenta weight percentile when adjusting for gestational age according to national standards (**Table S1**; p=0.369). The control group demonstrated evidence of a healthy pregnancy: term deliveries and appropriate for gestational age infants.

As expected, at 6 months postpartum more adverse cardiovascular risk parameters were observed in the high-risk group (**Table 4.2**). Although we observed higher blood pressures in the low-risk group (p=0.073, p=0.020), we note that a significant proportion of high-risk participants were still using antihypertensive medications at this time (**Table 4.2**; p=0.016). A significant increase in breastfeeding was also observed in the low-risk group (**Table 4.2**; p=0.013), in line with its reported cardioprotective effects [34]. When these same parameters were examined stratified by pregnancy outcome, PE women in general had significantly higher systolic blood pressures (p=0.027), diastolic blood pressures (p=0.005), total cholesterol levels (<0.001), low-

density lipoprotein levels (p=0.009), and triglyceride levels (p=0.048) in the postpartum period (**Table S2**).

3.2 FLT-1 and ENG Expression

Graphical representation of FLT-1 and ENG expression by lifetime cardiovascular risk status and pregnancy outcome can be found in **Figure 4.4**. No significant differences in either FLT-1 or ENG intensity were found between the high and low CVD risk groups (**Figure 4.4 panels A and D**; p=0.581, p=0.503). Although insignificant, a slight increase in average FLT-1 expression was observed in the high-risk group (99.65 [73.06, 119.96] vs 92.34 [64.18, 111.38]). A significant difference in FLT-1 intensity was observed when data was stratified by pregnancy outcome (**Figure 4.4 panel B**; p<0.001), with an increase in expression observed in PE cases (102.75 [91.24, 118.54] vs 58.62 [53.89, 67.89]). No significant differences were found in ENG expression by pregnancy outcome (**Figure 4.4 panel D**; p=0.629).

3.3 CD68+ Immune Cells

Graphical representation of CD68+ cells per villi area by lifetime cardiovascular risk status and pregnancy outcome can be found in **Figure 4.4**. No significant differences were found in CD68+ cells between the high and low CVD risk groups (**Figure 4.4 panel E**; p=0.261). However, although insignificant, a slight decrease in CD68+ cells was observed in the high-risk group (2466.54 [1502.63, 3510.21] vs 3098.94 [1842.26, 4331.89]). A significant difference in CD68+ cells was observed in the PE group compared to the control (**Figure 4.4 panel F**; 3098.94 [1845.48, 3910.62] vs 831.98 [257.69, 2302.85], p= 0.003) when data was stratified by pregnancy outcome.

3.4 CVD Risk Modelling

Results from the ROC analysis for the 6 models are presented in **Figure 5.5**. Individually, none of the three protein markers were found to be significantly associated with high-risk screening for lifetime CVD at 6 months postpartum (**Figure 4.4**). Although, when combined in a logistic regression model, the presence of increased FLT-1 and decreased CD68+ expression was moderately predictive of elevated CVD risk [**Figure 4.5 panel 4**; AUC 0.70 (0.54-0.87); sensitivity = 44.44%; specificity = 90.48%]. Further, significant improvements to model performance were achieved when clinical and placenta pathology data (presented in Chapter 3/Manuscript 2) were included (**Figure 4.5 panel 6**; AUC 1.00 (1.0-1.0); sensitivity = 100%; specificity=100%). In this model all patients were correctly grouped by lifetime cardiovascular risk estimates.

Table 4.1. Baseline Characteristics by Estimated Lifetime Cardiovascular Disease Risk Score.
Presented as median [IQR] or n (%). Significance evaluated at p<0.05.

	High-Risk (n=19)	**Low-Risk (n=22)**	***P*-value** (Mann-Whitney U, X^2)
PE diagnosis (%)	18 (94.7)	17 (77.3)	0.115
Postsecondary education (%)	16 (88.9)[a]	21 (85.5)	0.433
Married or common law (%)	19 (100)	22 (100)	-
Nulliparous (%)	10 (52.6)	13 (59.1)	0.678
Pre-pregnancy BMI (kg/m^2)	26.3 [22.7, 34.4][a]	27.1 [21.9, 31.7]	0.903
History of smoking (%)	1 (5.3)	0 (0)	0.276
History of HDP (%)	6 (31.6)	2 (9.1)	0.070
History of PMD (%)	3 (15.8)	0 (0)	**0.053**
Family history of CVD[b] (%)	8 (42.1)	11 (52.4)[a]	0.516
Family history of PE (%)	2 (11.8)[c]	5 (26.3)[d]	0.271

BMI: body mass index; CVD: cardiovascular disease; IQR: interquartile range; PE: preeclampsia
*Includes coronary artery disease and cerebrovascular disease
[a] Data unknown for one participant.
[b] Includes coronary artery disease and cerebrovascular disease
[c] Data unknown for two participants.
[d] Data unknown for three participants.

Table 4.2. Delivery and Postpartum Characteristics by Estimated Lifetime Cardiovascular Disease Risk Score.

Presented as mean ± standard deviation, median [IQR], or n (%). Significance evaluated at p<0.05.

	High-Risk (n=19)	**Low-Risk (n=22)**	***P*-value** (T-Test, Mann-Whitney U, X^2)
At delivery			
Maternal age at delivery (y)	35.4 ± 5.4	32.5 ± 4.9	0.062
Systolic blood pressure (mmHg)	132.4 ± 16.3[a]	130.0 ± 19.0	0.457
Diastolic blood pressure (mmHg)	82 ± 9.4[a]	73.8 ± 8.3	0.461
Antihypertensive medication use[b] (%)	15 (78.9)	13 (59.1)	0.173
Pregnancy weight gain (kg)	10.8 [5.8, 17.7]	11.9 [9.2, 16.5]	0.967
Gestational age delivery (wks)	37.5 [34, 39]	39 [37, 40]	**0.047**
Delivery before 37 weeks gestation (%)	8 (42.1)	3 (13.6)	**0.040**
Cesarean section (%)	8 (42.1)	8 (36.4)	0.707
Female infant (%)	9 (47.4)	8 (36.4)	0.476
Birthweight (g)	2668.6 ± 932.1	3075.5 ± 928.2	0.119
Birth weight percentile (%)	31 [13, 43]	40 [8, 68.3]	0.433
Small for gestational age[c] (%)	4 (21.1)	6 (27.3)	0.644
Admission to NICU (%)	10 (52.6)	5 (22.7)	**0.047**
Placental weight (g)	338.4 ± 96.3[d]	412.9 ± 109.4	**0.037**
Placenta weight percentile (%)	6 [2, 31.5][d]	25.5 [5, 73.8]	0.102
HELLP syndrome (%)	1 (5.3)	1 (4.5)	0.915
At 6 months postpartum			
Systolic blood pressure (mmHg)	120.5 [111.3, 127]	111.5 [102, 121.3]	0.073
Diastolic blood pressure (mmHg)	82.0 ± 9.4	73.8 ± 8.3	**0.020**
Antihypertensive medication use (%)	5 (26.3)	0 (0)[d]	**0.016**
Breastfeeding (%)	9 (50.0)[a]	19 (86.4)	**0.013**
Total cholesterol (mmol/L)	5.6 ± 0.5	3.9 ± 1.0	**<0.001**
Fasting glucose (mmol/L)	4.7 [4.3, 5.1]	4.5 [4.3, 4.8]	0.282
High density lipoprotein (mmol/L)	1.4 ± 0.4	1.7 ± 0.4	0.167
Low density lipoprotein (mmol/L)	3.5 [3.2, 3.6][a]	2.1 [1.9, 2.9]	**<0.001**

C-reactive protein (mmol/L)	3.4 [1.0, 8.0]	2.1 [0.6, 9.0]	0.381
Triglycerides (mmol/L)	1.1 [0.7, 1.7]	0.8 [0.6, 1.1]	**0.043**

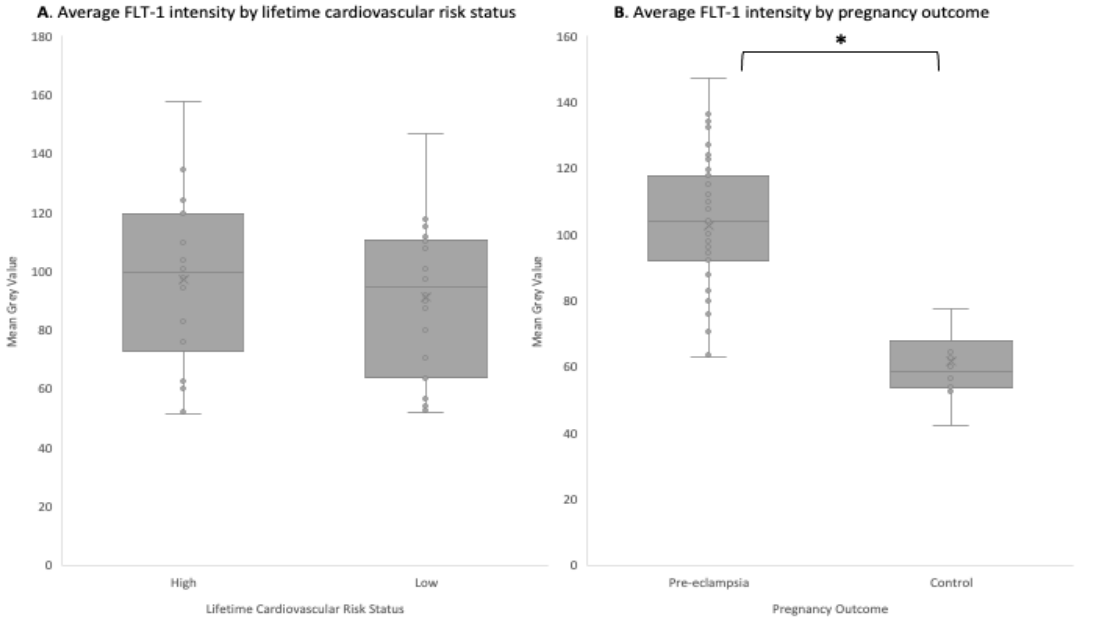
IQR: interquartile range; NICU: neonatal intensive care unit; PE: preeclampsia

[a] Data unknown for one participant.

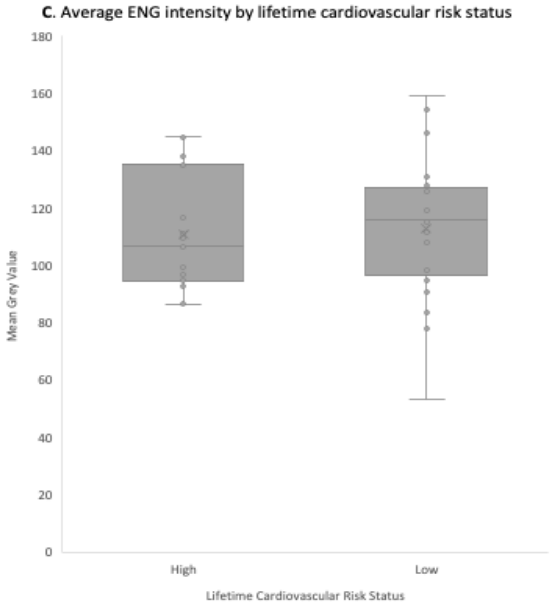
[b] Medication started during index pregnancy or postpartum prior to discharge from hospital

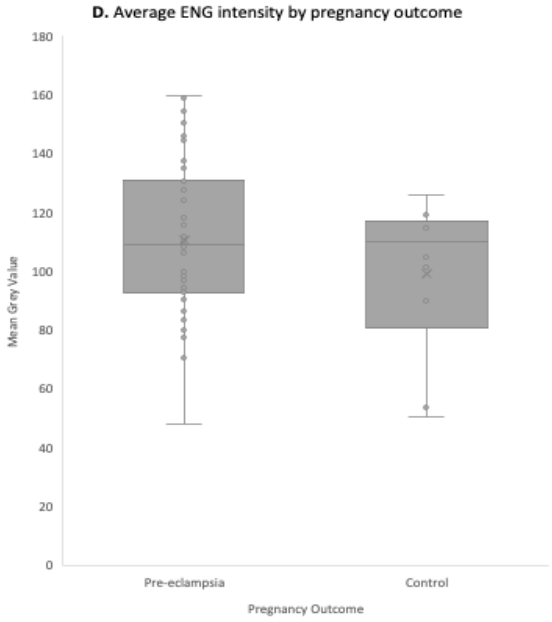
[c] Defined as <10th percentile.

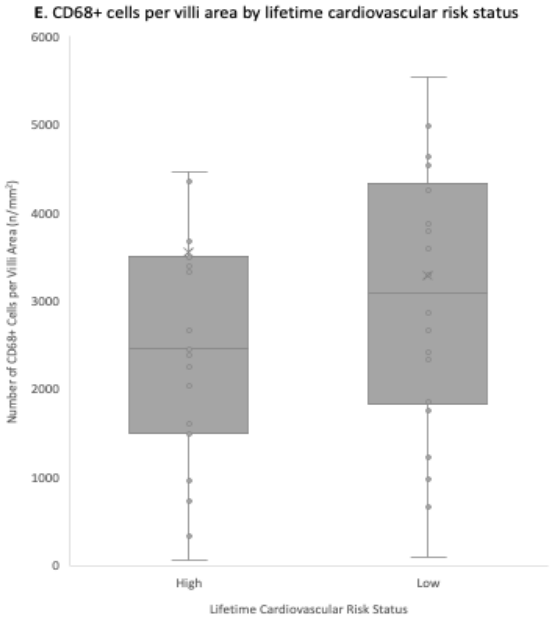
[d] Data unknown for two participants.


A. Average FLT-1 intensity by lifetime cardiovascular risk status
Mean Grey Value
180
160
140
120
100
80
60
40
20
0
High
Low
Lifetime Cardiovascular Risk Status

B. Average FLT-1 intensity by pregnancy outcome
*
Mean Grey Value
160
140
120
100
80
60
40
20
0
Pre-eclampsia
Control
Pregnancy Outcome


C. Average ENG intensity by lifetime cardiovascular risk status
Mean Grey Value
180
160
140
120
100
80
60
40
20
0
High
Low
Lifetime Cardiovascular Risk Status


D. Average ENG intensity by pregnancy outcome
Mean Grey Value
180
160
140
120
100
80
60
40
20
0
Pre-eclampsia
Control
Pregnancy Outcome


E. CD68+ cells per villi area by lifetime cardiovascular risk status
Number of CD68+ Cells per Villi Area (n/mm²)
6000
5000
4000
3000
2000
1000
0
High
Low
Lifetime Cardiovascular Risk Status

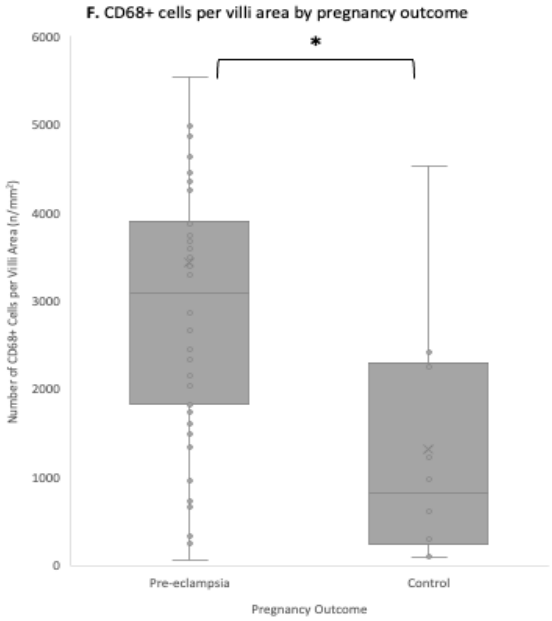

F. CD68+ cells per villi area by pregnancy outcome
*
Number of CD68+ Cells per Villi Area (n/mm²)
6000
5000
4000
3000
2000
1000
0
Pre-eclampsia
Control
Pregnancy Outcome

Figure 4.4 FLT-1, ENG, and CD68 Expression Stratified by Lifetime Cardiovascular Risk Status and Pregnancy Outcome. In the top panel, FLT-1 expression is presented by cardiovascular risk status (A) and by pregnancy outcome (B). In the following panel, ENG expression is presented by cardiovascular risk status (C) and by pregnancy outcome (D). In the last bottom panel, CD68+ cells per villi area is presented by cardiovascular risk status (E) and by pregnancy outcome (F). Sample size for comparisons by cardiovascular risk status is 41 and 58 by pregnancy outcome. Differences were evaluated by Mann-Whitney U Tests and significance was set at $p<0.05$. Significant differences between groups are indicated with a star.

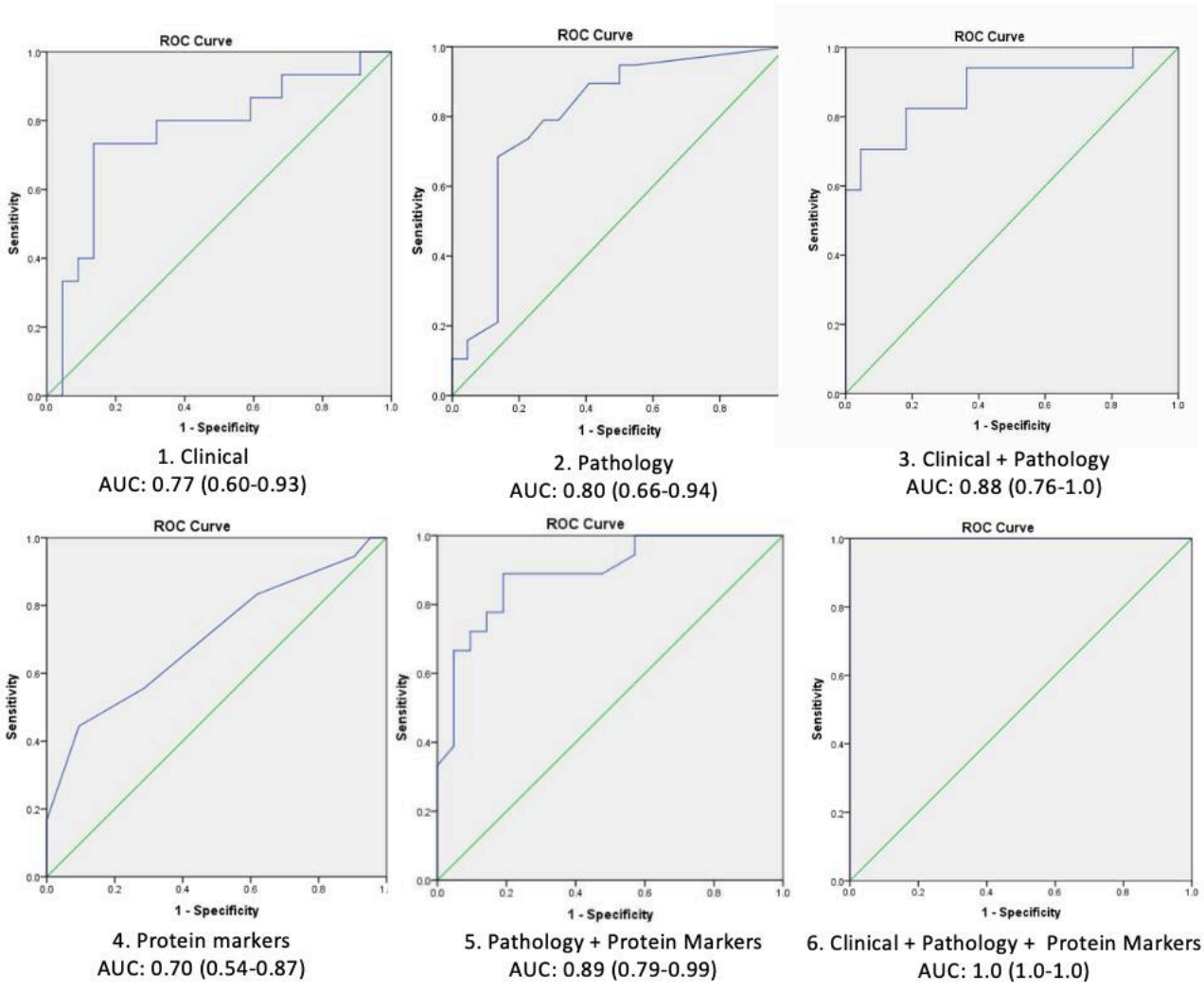


Figure 4.5 Area Under the Receiver Operator Curve for the Screening of High Lifetime Risk for Cardiovascular Disease at 6 Months Postpartum by 1. Clinical findings (maternal age, gestational weight gain, blood pressure at delivery, birth weight percentile, placenta weight percentile, and gestational age at delivery); **2.** Placental pathology (maternal vascular malperfusion severity score, chronic inflammation severity score, total pathology score, and absence of placental lesions); **3.** Clinical findings and placental pathology; **4.** Protein markers (elevated CD68+ cells per

villi area, elevated FLT-1, and elevated ENG); **5.** Placental pathology and protein markers; **6.** Clinical findings, placental pathology, and protein markers. Sample size was 41 for all models.

4. Discussion

Main Findings

In this study, no significant differences were found between individual placenta protein markers, associated with distinct forms of PE (FLT, ENG, CD68), according to estimated lifetime cardiovascular risk status in the postpartum period. However, when these same placenta proteins were combined in a multivariable model, increases in placental FLT protein expression combined with decreases in placental CD68 positive cells were moderately predictive of elevated lifetime risk for CVD. The predictive capacity of this model was further improved when clinical and placenta histopathology findings (originally presented in Chapter 3/Manuscript 2) were combined with placenta protein expression findings – capable of accurately predicting the postpartum CVD risk status of all patients in the current study. The cumulative severity of placental lesions and protein expression was important in our model, suggesting that critical thresholds of placental damage may reflect increased lifetime cardiovascular risk. Furthermore, as the clinical findings, placental histopathology, and placental protein expression used in our final model with 100% sensitivity and specificity are associated with the three distinct subclasses of PE, these subclasses may have differing postpartum CVD risk profiles. Although, this was unable to be further investigated in this present study.

Interpretation

Currently, there are no studies that attempt to assess CVD risk profiles by subclass of PE disease. As such, although this pilot study contained important limitations, it represents an

important first step in understanding the association(s) between PE and long term maternal cardiovascular health outcomes by comprehensively evaluating the combined predictive value of clinical findings, placental histopathology, and placental protein expression - variables that differ considerably according to PE subclasses. This provides a rationale for future, larger-scale longitudinal studies to further this understanding. Despite no studies directly focusing on PE subclasses, some studies have evaluated the association between individual subclass-associated features and elevated CVD risk. These findings are in line with results of this study.

Multiple studies report that in PE women with preterm deliveries, placental abruption, and small for gestational age infants, the risk for CVD is even higher [33]. In our cohort, we observed a significantly lower average gestational age at delivery in the high-risk group when compared to the low-risk. Although we did not observe any significant differences in birth weight percentile between the two groups, this variable contributed to the model with the best predictive performance for elevated cardiovascular risk.

Further, multiple studies have demonstrated that MVM lesions in the placenta are associated with altered cardiometabolic health following PE from 6-months to 10 years postpartum [23,24; Chapter 3/Manuscript 2]. In a previous study carried out with participants included in this cohort, a significant difference in MVM lesion severity was found between the high- and low-risk group [25; Chapter 3/Manuscript 2]. Thereby, we included this parameter in our logistic regression modelling, contributing to the best predictive performance of the model for elevated cardiovascular risk.

Elevations in anti-angiogenetic factors in PE pregnancies have likewise been associated with an increased postpartum CVD risk [35]. Of note, elevated sFLT-1 levels and decreased PIGF are

associated with signs of advanced arterial aging and persistent hypertension postpartum [35]. These findings have been replicated in a study assessing cardiovascular risk 12 years following PE pregnancy [35]. No studies have reported any associations thus far in peripartum sENG levels and cardiovascular risk in women with PE [35]. Although, both anti-angiogenic factors have been shown to influence vasodilatory and endothelial function in pre-clinical models of non-pregnant patients [35]. In PE women, these factors typically decrease in concentration within the maternal circulation following delivery (of the placenta), although recent studies demonstrate that in certain cases these imbalances may persist postpartum, indicative of continued maternal contributions to this anti-angiogenic pool [35]. This finding further highlights the heterogeneity of this disorder as well as a potential mechanism for the development of future CVD following PE [17,18]. In line with these findings, in the current study, a slight increase in average placental FLT-1 expression in the women who screened high-risk for CVD, although this association was insignificant in our pilot sample of reduced size. However, for placental ENG expression, this study was unable to find any significant differences by pregnancy outcome or by cardiovascular risk. As multiple studies have reported increased ENG expression in placentas from PE pregnancies when assessed by various methods including IHC and western blot, it is possible that these same results were not observed in this study as a result of the limited sample size [10]. Thus, future larger case-control and cohort studies should continue to explore its use as a potential marker for increased CVD risk following PE.

In the current pilot study a slight decrease in CD68 positive cells within placenta were observed in the high lifetime CVD risk group, although this finding was not significant – again likely the result of small sample size. However, it should be noted that this finding aligns with

previous findings from our group, which demonstrated slight reductions in inflammatory placental lesions in the high lifetime CVD risk group [25; Chapter 3/Manuscript 2]. Although significant differences in these lesions were not found individually between the high and low-risk groups, they did contribute to increasing the predictive performance of the developed model.

Based on the novel findings presented in this study and current literature, we cannot conclusively determine if different subclasses of PE have differing CVD risk profiles. To properly evaluate this, a robust longitudinal study is warranted. However, we note that the combination of clinical findings, placental histopathology, and placental protein markers- factors associated with distinct PE subclasses- were important variables to include in elevated CVD risk prediction models. Moreover, as MVM lesions, preterm birth, and sFLT-1 levels have been shown to correlate with altered cardiometabolic profiles in women with a history of PE, we hypothesize that women belonging to the 'Hypoxic PE' subclass may be those at highest risk for future CVD [17,18]. These three variables were included in our best performing predictive model. Conversely, placental and clinical findings consistent with the 'Inflammatory PE' subclass – specifically lesions of chronic inflammation, CD68+ immune cell staining and clinical findings of reduced fetal growth – appear to be more predictive of low CVD risk, possibly indicating that the CVD risk profile in this group of PE women is in fact no greater than the general pregnant population [17,18]. However, it should be noted that as this subclass is less common, it is very likely that the current study did not have a large enough sample size to accurately characterize subclass specific CVD risk profiles for this group [17,18]. Thus, future studies should focus on the CVD risk profiles associated with Inflammatory PE, as well as the idiopathic subclass of Maternal Maladaptation PE which was not specifically examined in the current study.

While our final model for elevated cardiovascular risk reported 100% specificity and sensitivity, repetition in a larger sample would likely increase patient heterogeneity, and as such may lead to a higher degree of data variability and possibly lower predictive performance of the developed model. However, this model should be further explored for its use a triage strategy in clinical settings where PE women with specific clinical and placental phenotypes – specifically those with MVM lesions and increase FLT-1 expression - could be prioritized for postpartum CVD risk screening. This strategy has the potential to improve the resource and financial burden that CVD risk screening for this population has on our healthcare system. Likewise, investment in these types of screening strategies could reduce future CVD morbidity and mortality, as it is preventable in 80% of cases [36].

Strengths and Limitations

Our study included several important strengths and limitations. A major strength of this study was our prospective study design in a major hospital within an urban Canadian population. In addition, our study included a control group with confirmed healthy pregnancies, although quite small in size. As studies on placental pathology are often retrospective, the inclusion of an appropriate control group is often a considerable limitation, as placentas from these pregnancies are not typically sent for pathological evaluation [37]. Further, we excluded participants with a history of chronic diseases associated with CVD risk to reduce selection bias. An additional strength was the choice of lifetime risk scores to evaluate CVD risk, as this assessment tool has also been demonstrated to be most appropriate for our study population - women of reproductive age - when compared to shorter-term 10-year and 30-year risk scores [27; Appendix A]. In addition, the high-risk threshold of 39% was determined in a population of women with a

history of PE and age-matched healthy controls [27]. Moreover, the final model includes a comprehensive assessment of variables associated with distinct forms of PE: clinical findings, placental pathology, and protein expression [17,18,25]. Although, despite the fact that this study was not adequately powered to detect any individual differences in these parameters, when combined in this model, sensitivity and specificity for CVD risk prediction were 100%.

The major limitation of this pilot study was the small sample size. Due to the prospective nature and subsequent 6-month follow-up appointment in the postpartum period, challenges were encountered related to participant attrition for follow-up assessments. As such, the final sample size for the logistic regression modelling was 41. A post-hoc analysis determined that in order to allow for at least 10 patients of each previously described PE subclass, a sample size of at least 91 participants would be required – based on observed proportions of Hypoxic PE, Inflammatory PE, and Maternal Maladaptation PE in a previously completed robust microarray study carried out by our group [17,18].

An additional limitation is the use of IHC to assess placental protein expression. The finding of increased FLT-1 and ENG expression in 'Hypoxic PE' was originally characterized using gene expression analysis. The original study design included the collection of flash frozen participant placenta samples, such that definitive PE subclass identification could be performed via quantitative polymerase chain reaction analysis as previously described [17,18]. However, challenged related to study recruitment, retention, and sample collection – due to staffing limitations at The Ottawa Hospital - inhibited the ability to proceed with this gold standard evaluation of PE subclass-specific genes of interest. However, although subclass-specific differences in protein expression have yet to be validated, the use of IHC to quantify these

markers could be more easily translated to clinical practice, as this tool is readily available and commonly used in pathology laboratories [37]. The case for IHC methodology of placental CD68 cell positivity is in fact stronger, as several studies have previously demonstrated that IHC can be used to detect differences in immune cell populations between PE subclasses [22], with our group previously publishing data that demonstrates elevated CD68+ cell infiltration into the placenta specifically from pregnancies confirmed to belong to the "immunologic subclass" of PE [22].

A final limitation to this study was the use of automated analysis tools to quantify expression of placenta protein markers. Although these tools made for more efficient analysis, an inherent error is associated with their use. More specifically, as noted in **Figure 4.2 panel B** some area outside the syncytiotrophoblast region was selected when creating the binary mask with the thresholding tool. More or less of this region was selected in certain images based on villi density and image contrast. Although this error is important to consider, the average grey value was obtained for this area rather than the total amount of staining to account for these differences. Likewise, as seen in **Figure 2.3**, some CD68+ cells were not accounted for with the automated cell detection feature. Furthermore, as more tissue area can identify more positive cells, the total number of positive cells was normalized for villi tissue area obtained with thresholding in ImageJ. As this analysis focused on the selection of complete villi structures, rather than the specialized syncytiotrophoblast region in FLT-1 and ENG stained samples, less difficulty was encountered in accurately defining this area of interest with the threshold tool. While errors are associated with these automated analysis tools, the alternative - manual cell counting- is also influenced by human error and is typically done on a smaller tissue area for efficiency purposes and was thus not selected for this study. An additional strength for the CD68 analysis was measurement on the

full-tissue section, as this allowed for a more representative sample of the placenta. For FLT-1 and ENG samples, the requirement to manually confirm area of interest thresholding required the use of an analysis protocol that balanced this resource limitation with the need to adequately account for possible tissue heterogeneity of protein expression, and as such the collection of 4 random snapshots at 20X magnification were collected from each full-tissue section for analysis t.

Finally, although the use of lifetime CVD risk scores has been validated in women of reproductive age, these tools were initially developed for use with an older and primarily biologically male population. As such, these models may still underestimate the true risk in this younger and biologically female population. Improvements to these risk screening tools are highly warranted to improve their specificity to a high-risk, young, female population. Further, studies measuring arterial health, the gold standard for CVD risk, should also be explored within this population to further understand the relationship between distinct forms of placental disease in pregnancy and elevated risk for CVD.

5. Conclusion

Placental protein expression profiles for FLT-1, ENG, and CD68, when combined with clinical and placenta histopathology data is capable of accurately predicting lifetime CVD risk estimates at 6 months postpartum. The results of this study demonstrate an important proof of concept that detailed examination of the placenta at the time of delivery following a pregnancy complicated by PE may provide a unique modality to identify women who would benefit most from referral to specialized postpartum cardiovascular screening clinics and early intervention.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Boards (or Ethics Committees) of the University of Ottawa Research Ethics Board (Protocol # A04-16-05; April 2016), Ottawa Health Science Network (Protocol # 20150941-01H; May 2016), and the Children's Hospital of Eastern Ontario (Protocol # 15/166X; January 2016).

Informed Consent Statement: Informed consent was obtained from all participants involved in the study.

Data Availability Statement: Reasonable requests for data will be considered.

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Conflicts of Interest: The authors declare no conflict of interest.

Supplementary Tables

Table S1. Baseline Characteristics by Pregnancy Outcome.
Presented as median [IQR] or n (%). Significance evaluated at p<0.05.

	Preeclampsia (n=35)	**Control (n= 6)**	***P*-value** (Mann-Whitney U, X^2)
Post-secondary education (%)	31 (91.2)[a]	6 (100)	0.449
Married or common law (%)	35 (100)	6 (100)	-
Nulliparous (%)	22 (62.9)	1 (16.7)	**0.035**
Pre-pregnancy BMI (kg/m^2)	27.3 [22.6, 35.1][a]	25.7 [22.2, 27.0]	0.161
History of smoking (%)	1 (2.9)	0 (0)	0.675
History of hypertensive disorders of pregnancy (%)	7 (20.0)	1 (16.7)	0.849
History of placenta-mediated diseases (%)	3 (8.6)	0 (0)	0.456
Family history of CVD[b] (%)	16 (47.1)[a]	3 (50.0)	0.894
Family history of PE (%)	6 (20.0)[c]	1 (16.7)	0.851

BMI: body mass index; CVD: cardiovascular disease; IQR: interquartile range; PE: preeclampsia
[a] Data unknown for one participant.
[b] Includes coronary artery disease and cerebrovascular disease
[c] Data unknown for five participants.

Table S2. Delivery and Postpartum Characteristics by Pregnancy Outcome.
Presented as mean ± standard deviation, median [IQR], or n (%). Significance evaluated at p<0.05.

	Preeclampsia (n=35)	**Control (n= 6)**	***P*-value** (T-Test, Mann-Whitney U, X^2)
At delivery			
Maternal age at delivery (y)	33.9 ± 5.7	34.7 ± 3.8	0.669
Systolic blood pressure (mmHg)	135.7 ± 16.6	106.2 ± 9.1[a]	**<0.001**
Diastolic blood pressure (mmHg)	85.0 ± 10.5	69.4 ± 6.9[a]	**0.003**
Antihypertensive medication use[b] (%)	28 (80.0)	0 (0)	**<0.001**
Pregnancy weight gain (kg)	11.7 [8, 17.4]	11.9 [10.4, 16.5]	0.894
Gestational age delivery (wks)	38 [34.3, 39]	40 [39.8, 40.3]	**0.004**
Delivery before 37 weeks gestation (%)	11 (1.4)	0 (0)	0.108
Cesarean section (%)	14 (40.0)	2 (33.3)	0.757
Female infant (%)	13 (37.1)	4 (66.7)	0.175
Birth weight (g)	2710.5 ± 926.7	3746.0 ± 386.6	**<0.001**
Birth weight percentile (%)	30 [8, 48.8]	67 [48.8, 81.5]	**0.016**
Small for gestational age[c] (%)	10 (28.6)	0 (0)	0.132
Admission to NICU (%)	15 (42.9)	0 (0)	**0.044**
Placental weight (g)	369.1 ± 111.6	453.3 ± 39.9	**0.003**
Placenta weight percentile (%)	8 [2.5, 41][d]	29 [9.3, 73.8]	0.369
HELLP syndrome (%)	2 (5.7)	0 (0)	0.548
At 6 months postpartum			
Systolic blood pressure (mmHg)	117 [111, 126]	103.5 [95.8, 113.5]	**0.027**
Diastolic blood pressure (mmHg)	78.4 ± 9.1	68.2 ± 5.8	**0.005**
Antihypertensive medication use (%)	5 (9.6)[d]	0 (0)	0.299
Breastfeeding (%)	22 (64.7)[a]	6 (100)	0.082
Total cholesterol (mmol/L)	4.9 ± 0.9	2.4 ± 0.5	**<0.001**
Fasting glucose (mmol/L)	4.7 [4.3, 5.1]	4.5 [4.3, 5]	0.384
High density lipoprotein (mmol/L)	1.5 ± 0.4	1.8 ± 0.5	0.294
Low density lipoprotein (mmol/L)	3.0 [2.1, 3.5][a]	1.9 [1.7, 2.3]	**0.009**
C-reactive protein (mmol/L)	2.9 [0.8, 8.4]	1.6 [0.7, 12.8]	0.518
Triglycerides (mmol/L)	1.0 [0.7, 1.5]	0.5 [0.4, 1.3]	**0.048**
High-risk for lifetime CVD (%)	18 (51.4)	1 (16.7)[e]	0.115

CVD: cardiovascular disease; IQR: interquartile range; NICU: neonatal intensive care unit; PE: preeclampsia

[a] Data unknown for one participant.

[b] Medication started during index pregnancy or postpartum prior to discharge from hospital

[c] Defined as <10th percentile.

[d] Data unknown for two participants.

[e] Data unknown for three participants.

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Chapter 5

Discussion

5.1 General Discussion

The overarching hypothesis of this MSc. thesis was that standardized and detailed evaluation of the placenta at the time of delivery - specifically the presence of distinct histopathology lesions and placenta protein markers - can contribute to the identification of women at high-risk for CVD following a pregnancy complicated by placenta mediated disease. The thesis first presents a comprehensive systematic review describing and comparing current knowledge on postpartum CVD risk estimates associated with a number of different PMDs (Chapter 2/Manuscript 1). This body of work emphasizes the importance of physician education, patient counselling and need for postpartum cardiovascular health screening for women following all types of PMDs. Further, this review highlights the pressing need for more research on best screening practices and risk stratification triage strategies for this unique patient population. In line with these recommendations, Chapters 3 and 4 (Manuscripts 2 and 3) present cohort studies specifically aimed at determining if detailed placental evaluation at the time of delivery might offer a novel triage strategy to identify women at highest risk for future CVD following a pregnancy complicated by PE. These proof-of-concept studies nicely demonstrate that specific placental lesions (I.e. lesions of maternal vascular malperfusion) and placenta protein expression profiles (I.e. high FLT expression and low CD68+ cell counts) are indicative of high lifetime CVD risk, particularly when combined in a predictive model that includes basic clinical information (I.e. gestational age at delivery, maternal BMI etc) that is also available at the time of delivery. While not conclusively determined in the completion of this thesis, these results certainly suggest that women presenting with the “hypoxic” subclass of PE – women who demonstrate the unique placental findings consistent with high CVD risk - might be those at

highest risk of compromised cardiovascular health and may benefit the most from referral to specialized postpartum screening clinics and early intervention.

In Chapter 2 (Manuscript 1), we explored the association between PMD type and estimated CVD risk. We observed that all forms of PMD demonstrated evidence of heighted cardiovascular disease risk in the postpartum period. Notably, systolic blood pressure, BMI, current smoking, and total cholesterol were all non-optimal in at least one study of each PMD type. As such, this evidence demonstrates that a revision of current CVD risk assessment guidelines warrants a revision and should include all forms of disease by including small for gestational age, stillbirth, and placental abruption. This body of work also highlighted the pressing need to carry out focused research on associations between less studied PMDs and maternal CVD risk – namely, following pregnancies complicated by preterm birth, stillbirth and placental abruption. In parallel, mechanistic studies aimed at teasing apart the biological underpinnings of these relationships are required such that effective therapeutic and/or behavioural interventions can be identified to improve maternal cardiovascular health following a PMD pregnancy.

In Chapter 3 (Manuscript 2), we explored if specific placental histopathological lesions were associated with elevated cardiovascular risk at 6 months postpartum following a pregnancy complicated by PE – a common and serious PMD. We observed that PE women with elevated lifetime risk for CVD demonstrated an overall increase in placenta pathology, as indicated through an increase in total number of placental lesions observed. More specifically, we found that women at highest lifetime risk for CVD demonstrated an increase in number and severity of lesions belonging to the category of MVM. This is in line with previous studies where MVM lesions

in normotensive and pathological pregnancies are associated with altered cardiometabolic health 8-10 years postpartum [16]. Furthermore, severity of MVM lesions was moderately predictive of elevated cardiovascular risk as demonstrated in our logistic regression model.

MVM lesions represent abnormalities in the maternal spiral arteries and subsequent damage to the placenta parenchyma consistent with hypoxic, ischemic, or oxidative environments [65]. These lesions have been associated with PE cases, but placental pathology findings are heterogeneous for this disorder with minimal and inflammatory patterns as well [34,35]. MVM lesions can also be observed in other types of PMDs, namely fetal growth restriction and spontaneous pre-term birth [65]. The occurrence of these lesions in the placenta may indicate that abnormalities in the maternal vasculature were present prior to conception [65]. In studies that prospectively assess cardiometabolic health from pre-pregnancy to postpartum, increased cardiovascular risk appears to be associated with PMDs even after adjusting for certain pre-pregnancy CVD risk indicators [65]. It is important to note however that these studies are not without limitations and that evidence of sub-clinical CVD is reported in women who then develop PMDs during pregnancy and likewise in the postpartum period following a PMD complicated pregnancy in previously healthy women [39,65]. Thus, more studies are needed to further our understanding of the disease mechanisms underlying the association between PMDs and CVD. As the placenta provides a snapshot into peripartum health, it can provide information on the subtype of PMD occurring [65]. The presence of MVM lesions may then classify a subset of women with a prominent utero-placental vascular disorder that are of increased risk for CVD. As the placenta is easily assessable after delivery and its pathological

evaluation is recommended in cases of PMD, it could offer for an efficient, cost-effective triage strategy for CVD risk screening assessments.

In Chapter 4 (Manuscript 3), we furthered this study by performing IHC analysis for FLT-1, ENG, and CD68 expression in placental samples from a subset of the cohort described in Chapter 3 (specifically, the Ottawa recruited subset of this cohort). Each protein marker on its own was not found to be significantly different according to estimated maternal CVD risk group, however when used in combination, these proteins were moderately predictive of elevated cardiovascular risk, with results comparable to the predictive accuracy associated with placental histopathology and clinical findings alone. Importantly, when we developed a model in which all three feature sets were included (clinical data, placenta histopathology and placenta protein expression) we observed a very strong predictive accuracy in the identification of women at highest risk for CVD in the future. Collectively, this body of work highlights the clinical utility of exploiting unique placental features at the time of delivery to identify high-risk women for CVD following PE.

5.2 Clinical Impacts

Based on the results presented in these three manuscripts, the relationship between the placenta and future CVD risk is further understood. As demonstrated in our systematic review (Manuscript 1/Chapter 2), all types of PMDs demonstrated signs of altered CVD risk profiles at varying times postpartum. For most CVD risk markers, non-optimal averages were found in the early postpartum period and remained relatively stable in the long-term. As such, our results continue to support the possibility of performing CVD risk assessments in the early postpartum period. In addition, this timepoint typically involves high interaction with the healthcare system

and high motivation for lifestyle changes [59]. Thus, CVD risk assessments surrounding delivery may be a novel and cost-effective method to risk stratify women with a history of PMDs.

Further, current standard of practice for histopathological evaluation recommends submission of placental samples for pregnancies complicated by PE as well as other PMDs. As such, based on the results presented in Manuscript 2 /Chapter 3, both placental histopathology and clinical findings collected at delivery could be used in a risk prediction model to identify women with an elevated risk for CVD. We note that these proof-of-concept studies only focused on women with a history of PE, however due to the shared PMD mechanisms and placental pathology findings, we postulate that this risk prediction model could be adapted for all types of PMDs. Further, MVM lesions, those associated with elevated risk for future CVD, are a common finding in all types of PMDs [65]. It must be noted, however, that these types of evaluations require the collection of a complete and standardized dataset of placental features. Currently, standard clinical practice only includes the generation of a narrative report by the perinatal pathologist, methodology which has been demonstrated to results in incomplete and highly variable outputs. Working alongside our pathologist colleagues, our group has developed a comprehensive synoptic reporting tool, which requires the pathologist to input an absent/present/severity score for every possible placenta lesion. We have successfully validated this tool and demonstrates it clinical utility in the generation of comprehensive and reproducible results. However, this reporting format likely will increase pathologist workload and as such implementation into the clinical environment will require buy in from hospital administrators, departments heads and pathologists alike. It should be noted, however, that the field of onco-

pathology has successfully implemented a similar synoptic reporting tool across all of North America, with tremendous clinical and research-endeavour success [9].

This body of work also sought to assess whether mothers with distinct subclasses of placental disease, specifically in the context of PE, demonstrate an elevated risk for CVD in the postpartum period. In order to examine this, the use of an IHC panel to identify proteins unique to PE subclasses was implemented. While we were able to demonstrate success in the identification of differential risk postpartum CVD risk profiles, the added benefit of this IHC panel was minimal compared to a model that used just histopathology and clinical datasets. As the IHC panel is not part of the current standard of care for these patient populations, an in-depth cost-benefit analysis would be required to determine if these additional tests would be warranted in the context of post-partum screening protocols. While we acknowledge that these additional analyses are unlikely to identify this panel as an economically feasible screening tool in a clinical setting, this work does provide us with very important information regarding potential underlying disease mechanisms that might be intimately connected to lifelong CVD risk in the mother. Specifically, the subclass of PE that demonstrated evidence of poor utero-placental perfusion and subsequent placental hypoxic disease seem to be at highest risk, and hence studies focused on understanding feto-placental-maternal crosstalk in instances of poor placental perfusion should be highly prioritized.

What has become abundantly clear in the review of the literature and the execution and dissemination of this body of work, is that widespread education initiatives aimed at health care providers and patients alike are warranted, with the explicit goals of improving the level of knowledge of regarding future maternal CVD risk following PMDs. These efforts should include

education on placental pathology submission practices, the value of placental pathology findings, and the resources available for women who screen high-risk for CVD. Further, clinical guidelines for CVD risk assessments should be updated to include all types of PMDs.

5.3 Study Limitations

While this thesis presents several impactful results, there are some study limitations that warrant consideration in the interpretation of findings. Limitations of each individual manuscript are discussed in detail in each respective chapter. To summarize, the main limitations of Manuscript 1/Chapter 2 were differences in risk assessment timing, correlation-based nature, co-morbidity of many PMD conditions, lack of diversity in study population, and disproportional spread of PMD types. For Manuscript 2/Chapter, 3 they were differences in study sampling methods between study sites, study power, accuracy of placental sampling, and method of CVD risk estimation. For Manuscript 3/Chapter 4, they were study power, automatic quantification, and method of CVD risk estimation.

Overall, the principal limitations to this thesis are as follows. First, challenges in sample size and study power were observed in all manuscripts. In the systematic review, a large number of studies was identified, however there were disproportionally more studies on PE and less on preterm birth, stillbirth, and placental abruption. In our 2 cohort studies, we endured challenges with recruitment and participant retention for the follow-up in the postpartum period. As such, we were underpowered to detect differences in all variables assessed. Despite this, we were powered for some variables and our results lay the foundation for future larger-scale studies. Second, the use of lifetime CVD risk assessments was a limitation across all manuscripts. Although these can provide an idea of the current cardiovascular function and identify potential risk

factors, these models were originally developed with a primarily older, male sample and thus underestimate the risk present in younger females of reproductive age [51]. Although, this method of risk assessment has been shown to be the most appropriate for this population compared to shorter-term risk scores. In addition, the threshold of 39% for the estimation of high-risk was developed specifically within a population of women with a history of PE and healthy female controls of reproductive age [51]. However further research on sex-specific risk estimation models is highly warranted [51]. Moreover, improved measures of risk assessment should be considered, including direct measurements of arterial health in the postpartum period, where arterial stiffness, arterial hemodynamics, and endothelial function are measured [45,46]. Ongoing work by our group is expanding the results presented in this thesis to examine the relationship between placental histopathology findings and these more direct measurements of postpartum vascular health following a PE pregnancy. Third, there is a legitimate concern that current interventional options for women who are identified at high risk in the postpartum period are limited to behavioural interventions at this time. While these have been shown to be highly effective when properly implemented, new parents may find behavioural interventions that require significant time and/or financial commitments to be a challenge [59]. As such, research on effective interventions specific to younger women – particularly those with young infants/children - at increased risk for CVD is highly warranted.

5.4 Future Directions

Currently, a follow-up study is ongoing with the original DREAM cohort participants (data presented in manuscripts 2 and 3). Here, participants are undergoing an arterial health assessment directly, the gold standard for estimating future cardiovascular risk. From this,

endothelial dysfunction, arterial stiffness, and arterial hemodynamics are estimated from a combination of arterial tonometry and imaging techniques [45,46]. This method can detect sub-clinical atherosclerotic disease [45,46]. Currently, 8 assessments on participants with a past PE pregnancy have been completed. In addition, our research group has previously generated a machine-learning model to automatically detect MVM lesions in the placenta [Unpublished]. As these lesions are associated with elevated cardiovascular risk [Manuscript 2/Chapter 3], automatic detection and computer modelling for cardiovascular risk will be explored in future studies. Further research avenues could evaluate the cost-benefit analysis of the use of placental pathology in predicting cardiovascular risk. An updated recommendations for current clinical practice guidelines is also warranted based on multiple papers published in the recent years on MVM lesions and elevated risk for CVD [16]. As highlighted by our systematic review, more research is also needed on preterm birth, stillbirth, and placental abruption. Finally, exploration into the subclasses of PE and other PMDs should continue to further understand the complex etiology of these conditions.

5.5 Interdisciplinary Perspective

This MSc thesis embodies the true spirit of interdisciplinary health research – bringing together a unique group of clinicians and researchers with diverse expertise to identify pressing and novel research questions, collectively design and execute a series of clinical and basic science studies, and jointly integrate and interpret the collected research findings with the shared goal of improving life-long cardiovascular health of women – a population with unique risk factors and clinical needs that have historically been largely ignored. This project sits at the intersection of

several research and clinical domains, including: perinatal pathology, cardiology, obstetrics, placental biology, molecular biology, and disease prediction modelling.

In the completion of the systematic review presented in Chapter 2, collaboration with a health sciences research librarian was necessary to generate a strong search strategy and study protocol aimed at answering our collective research question: “What is the association between placenta-mediated diseases and estimated maternal risk for CVD?”. Our protocol, search, and results were reviewed by experts in placenta biology and maternal cardiovascular disease risk estimation for content accuracy and identification of real gaps in knowledge in this area. Armed with this knowledge, our interdisciplinary group worked together to design and implement two studies across two clinical sites to assess if specific forms of placental damage and/or protein markers of this damage may help in the identification of high-priority women who would benefit most from postpartum screening and follow up (Chapters 3 and 4). These studies were driven by identified gaps in knowledge, coupled with the challenges faced by clinicians surrounding how to ensure that these women are properly referred and seen following delivery. In the completion of these studies, we worked alongside our partners in two multidisciplinary postpartum maternal health clinics. These novel clinics are a result of advancements in the emerging field of cardio-obstetrics, where women with pregnancy-related cardiovascular risk indicators can receive a complete cardiovascular risk assessment in the postpartum period. The establishment and appropriate implementation of these clinics are extremely important, as they help fill an important gap in women’s health care – women’s heart health [59]. The completion of these studies also necessitated the expertise of perinatal pathologists and placental biologists – ensuring that all relevant forms of placental disease and damage were identified and recorded

appropriately. Finally, predictive modelling and general statistical analysis was carried out through collaborations between biostatisticians, obstetricians, and cardiologists – ensuring that the models developed were appropriately designed and clinically relevant. Without this diverse team of experts working together towards the common goal, the findings and impact of these studies would not be possible.

Thus, although the background, methodology, and interpretation of results involved understanding unfamiliar domains of study, the overall impact of this interdisciplinary project was highly beneficial. Through the collaboration of pathology, obstetrics, cardiology and biology, the understanding of the role of the placenta in predicting future CVD was furthered in ways that would otherwise not be possible. Collaborations such as these should continue in future projects to improve the understanding of long-term health outcomes following placenta-mediated diseases, notably future maternal CVD morbidity and mortality.

5.6 Conclusions

In conclusion, our objective of evaluating the use of the placenta to predict future CVD following PMDs was achieved. Although, this thesis presents preliminary evidence of this relationship and is not without its limitations, it offers the foundation for future larger scale studies. To resume, in our comprehensive review of the literature, we evaluated the association between various PMDs and CVD clinical and biochemical markers as well as estimated CVD risk scores. It was found that, all form of PMD demonstrated evidence of increased CVD risk markers. Notably, all PMD types displayed at least one study with non-optimal systolic blood pressure, BMI, and total cholesterol. To test our main hypothesis, we then evaluated the association of placental histopathology lesions and elevated lifetime CVD risk in women with a history of PE.

Here, we found that MVM lesions were associated with a 3-fold increase in elevated lifetime CVD risk. In our multivariate regression modeling, we found that placental histopathology alone was moderately predictive of elevated CVD risk but that its predictive accuracy was greatest when basic clinical and placental histopathology findings were combined (respective AUC: 0.63 and 0.73). We then furthered this work by assessing the relationship between placental protein expression and elevated CVD risk in women with a history of PE. Here we found no associations between expression of individual placental proteins FLT-1, ENG, or CD68 and elevated CVD risk. However, when all three proteins were combined in a multivariable linear regression modelling, we found increased performance we demonstrated moderate predictive capabilities in identifying high risk women. Notably, when this model was complemented with the addition of clinical and placental histopathology feature sets, predictive performance of this model dramatically increased – demonstrating an and placental protein expression had an AUC of 1.0. Thus, this thesis provides compelling proof-of-concept results indicative of the clinical utility of detailed placenta examination in predicting maternal lifetime CVD risk status following PMDs of pregnancy.

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Appendices

Appendix A. Supplementary Figures.

A.1. Synoptic Reporting Tool for Placenta Pathology Examinations

Synoptic framework for placental pathology

Microscopic Lesions
Category 1 : Evidence of maternal vascular malperfusion
Placental infarct(s) • *Refer to gross description, exclude marginal infarctions in a term placenta* 0 = No 1 = Yes; Indicate if: □ Early (crowding/congestion of villi, may be hemorrhagic, early loss of stromal nuclear staining) OR □ Remote (necrotic changes, loss of trophoblast nuclear staining and ghost villi)
Distal villous hypoplasia • *Reduction in size of intermediate villi with dispersed terminal villi and reduced number that appear thin and elongated, widening of intervillous space; adjusted for gestational age; involves at least 30% of full thickness slide* 0 = Not present 1 = Focal (1 slide only) 2 = Diffuse (≥2 slides)
Accelerated villous maturation pattern • *Presence of term-appearing/hypermature villi for gestational age, not in areas adjacent to infarction* 0 = Not present 1 = Diffuse pattern (seen on 2 or more slides)
Increased syncytial knots • *Aggregates of syncytiotrophoblast nuclei along stem and/or at terminal villi involving 33% of the villi or more* 0 = Not increased 1 = Increased (defined as knots on more than 33% of the villi)
Villous agglutination • *Clusters of adherent terminal villi (>2, <20), enmeshed by fibrin and/or bridging syncytial knots and/or apoptotic debris assessed at 4-10X magnification* 0 = Not present 1 = Present, focal (present on 1 slide only) 2 = Present, present on 2 more slides
Category 2 : Evidence of maternal decidual arteriopathy
Insufficient vessel remodelling • *Unremodelled vessels with retained muscularis and elastin (capsularis) or any retained muscularis or elastin (basalis)* 0 = Not present 1= Present; Location: decidual capsularis or basalis or both
Fibrinoid necrosis 0 = Not present 1 = Present 2 = Present with atherosis (foamy histocytes); Location: capsularis or basalis or both
Category 3 : Implantation site abnormalities
Microscopic accreta 0 = Not present 1 = Present, basal plate with myometrial fibers with intervening decidua 2 = Present, basal plate with adherent myometrial fibers, ≤2 layers of decidual cells separating myometrium from anchoring villi/Rohr's fibrin 3 = Present, myometrial fibers directly adherent to Rohr's fibrin/anchoring villi (equivalent to pathologic accreta)

Version 1.9; December, 2018
Bainbridge and Grynspan

Synoptic framework for placental pathology

Category 4 : Evidence of ascending intrauterine infection
Maternal inflammatory response (exclude subchorionitis) **Stage:** 0 = Not present 0+: Neutrophils in subchorionic fibrin only1 = Stage 1 – neutrophils in trophoblast layer of membrane 2 = Stage 2 – diffuse or patchy neutrophils in fibrous chorion or amnion 3 = Stage 3 – amnion or chorionic plate necrosis
Grade: 0 = Not present 1 = Mild or moderate – lacks criteria for Grade 2 2 = Severe – confluent neutrophils between chorion and decidua, greater than 10 x 20 cells in extent with greater than 3 foci or a large continuous band
Fetal inflammatory response **Stage:** 0 = Not present 1 = Stage 1 – chorionic vessel vasculitis or umbilical venous vasculitis 2 = Stage 2 – umbilical vasculitis with umbilical arteritis 3 = Stage 3 – necrotizing funititis/concentric umbilical perivasculitis
Grade: 0 = Not present 1 = Mild to moderate – lacks criteria for Grade 2 2 = Severe – heavy inflammation of vessel within the umbilical cord or chorionic plate vessel with vessel wall damage
Thrombosis of any of the umbilical or chorionic fetal vessels present: Yes or no
Category 5 : Evidence of placenta villous maldevelopment
Chorangiosis • *Hypercapillarised terminal villi* 0 = Not present 1 = Present with >10 terminal villi with ≥10 capillaries, seen in 2 or more slides
Chorangiomas 0 = Not present 1 = Present and < 3 cm in size 2 = Present and ≥ 3cm in size or >5 total nodules
Delayed villous maturation[33] • *Monotonous villi (≥10) with centrally placed capillaries and decreased vasculosyncytial membranes resembling villi in early pregnancy, present in at least 30% of full thickness section* 0 = No villous immaturity 1 = Focal – lesion seen on one slide only 2 = Diffuse – seen on ≥2 slides
Category 6 : Evidence of fetal vascular malperfusion
Avascular fibrotic villi (requires the absences of VUE) 0 = None present 1 = Small foci – 3 or more foci of 2-4 terminal villi showing complete loss of villous capillaries and bland hyaline fibrosis of the villous stroma 2 = Intermediate foci – 3 or more foci of 5-10 terminal villi 3 = Large foci – 3 or more foci of >10 villi

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Synoptic framework for placental pathology

Thrombosis

0 = Not present
1 = Present
Location : Umbilical, chorionic plate, stem vessel
Number : _____ ; □ Occlusive OR □ Non-occlusive

Intramural fibrin deposition

- *Subendothelial or intramuscular fibrin or fibrinoid deposition within the wall of large fetal vessel (recent), with calcifications (remote)*

0 = Not present
1 = Recent, Number: _____
2 = Remote, Number:_____

Villous stromal-vascular karyorrhexis

- *≥3 foci of 2-4 terminal villi showing karyorrhexis of fetal cells with preservation of surrounding trophoblasts*

0 = Not present
1 = Present

Stem villous vascular obliteration

0 = No
1 = Yes

High-grade fetal vascular malperfusion

- *≥1 focus of avascular villi (≥45 cumulative avascular villi over 3 sections, average of ≥15 villi per section) with or without thrombus OR ≥2 thrombi in chorionic plate or major stem villi OR multiple non-occlusive thrombi*

0 = Not present
1 = Low-grade (does not achieve high-grade criteria); □ Segmented OR □ global
2 = High-grade; □ Segmented OR □ global

Category 7 : Evidence of utero-placental separation

Chorionic hemosiderosis

0 = No
1 = Yes

Presence of retroplacental adherent hematoma

- *Refer to gross description, confirm histologically; compressive overlying parenchyma*

0 = No
1 = Yes; Overlying infarction? Yes or No

Category 8 : Fibrinoid

Increased focal perivillous fibrin deposition (perivillous fibrin plaque)

- *Increased amounts of fibrin coating proximal stem villi and/or terminal villi*

0 = Not present
1 = Present; Percentage of total parenchyma occupied: _____ %

Massive perivillous fibrin deposition pattern

0 = Not present
1 = Diffusely present, 30-50% of intervillous volume, seen on at least 2 slides
2 = Diffusely present, >50% of intervillous volume, must be seen on all slides

Maternal floor infarct pattern

Histological confirmation : yes or no

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Bainbridge and Grynspan

Synoptic framework for placental pathology

Category 9 : Intervillous thombi
Intervillous thrombi 0 = Not present 1 = Present, confirmed histologically
Category 10 : Evidence of chronic inflammation
Villitis of unknown etiology 0 = Not present 1 = Low-grade, inflammation affecting <10 contiguous villi in any one focus, >1 focus * □ *Focal (1 slide only) OR* □ *multifocal (>1 slide)* * *Avascular villi: Yes or No* 2 = High-grade VUE – inflammation affecting >10 contiguous villi, seen in multiple foci on >1 section * □*Patchy (multiple foci, 1 with >10 contiguous villi) OR* □ *diffuse (>30% of all terminal villi involved)* * *Avascular villi: Yes or No*

A.2. Cardiovascular Risk Assessment.

Maternal Health Clinic Follow Up Form

LAST NAME	FIRST NAME	OHIP NUMBER	VERSION CODE	MOTHERS PROGRAM ID
DATE OF BIRTH (DD/MM/YYYY)	HOME TELEPHONE NUMBER	FAMILY PHYSICIAN		CR NUMBER
ADDRESS	UNIT NUMBER	CITY	PROVINCE	POSTAL CODE

Pregnancy-Related Cardiovascular Risk Indicators

Risk Indicator	Notes	Previous Pregnancy	Index Pregnancy
Preeclampsia	Preeclampsia, eclampsia or HELLP syndrome.		
Gestational Hypertension	Hypertension in pregnancy without proteinuria.		
Gestational Diabetes or Gestational Impaired Glucose Tolerance	Diagnosis of gestational diabetes is based on 2 or more abnormal values on a 75g Oral Glucose Tolerance Test. Gestational Impaired Glucose Tolerance is based on a single abnormal value on a 75g OGTT.		
Abruption	The occurrence of a clinically significant abruption leading to delivery or adverse maternal/fetal outcome.		
Excessive Weight Gain	Excessive weight gain during pregnancy indicates increased risk. It is determined based on the patient's pre-pregnancy (pp) BMI. The criteria are as follows; ppBMI <18.5 and >18.0 kg gained, ppBMI 18.5 - 24.9 and >16.0 kg gained, ppBMI 25.0 - 29.9 and >11.5 kg gained, ppBMI >29.9 and >9.0 kg gained.		
Preterm Birth	Preterm birth at <37 weeks gestation		
IUGR	Any birth <5th %tile for gestational age or term baby <2500g		
Total Number of Pregnancy Related Cardiovascular Risk Indicators			

History and Examination

	Risk Indicator		Notes	Risk Factor
1	**Age** (Years)		Current age. Risk increases with age.	
2	**Height** (cm)		Weight measured pre-pregnancy or during early pregnancy. BMI = (Weight/ (Height*Height))*10,000. Underweight BMI <18.5, Ideal BMI 18.5-24.9, Overweight BMI 25.0-29.9, Obese BMI >29.9.	
	Pre-pregnancy Weight (kg)			
	Pre-pregnancy BMI (kg/m^2)			
3	**Current Weight** (kg)		Weight at 6 months postpartum. BMI = (Weight/ (Height*Height))*10,000. Underweight BMI <18.5, Ideal BMI 18.5-24.9, Overweight BMI 25.0-29.9, Obese BMI >29.9.	
	Current BMI (kg/m^2)			
4	**Weight at Delivery** (kg)		Excessive weight gain during pregnancy indicates increased risk. It is determined based on the patient's pre-pregnancy (pp) BMI. The criteria are as follows; ppBMI <18.5 and >18.0 kg gained, ppBMI 18.5 - 24.9 and >16.0 kg gained, ppBMI 25.0 - 29.9 and >11.5 kg gained, ppBMI >29.9 and >9.0 kg gained.	
	Weight Gained in Pregnancy (kg)			
5	**Pregnancy Weight Retention** (kg)		At 6 months postpartum. Pregnancy Weight Retention = Current Weight – Pre-pregnancy Weight. It is recommended that women attempt to return to their pre-pregnancy weight by 6 months postpartum.	
6	**Current Waist Circumference** (cm)		At 6 months postpartum. Measure just above the uppermost lateral border of the right iliac crest. The plane of the tape should be parallel to the floor. The tape should be snug, but not compress the skin. Take measurement at the end of normal expiration.	
7	**Pre-pregnancy Blood Pressure** (mmHg)		Blood pressure taken pre-pregnancy or during early pregnancy. Systolic blood pressure greater than 130 mmHg or diastolic blood pressure greater than 85 mmHg indicates increased risk.	
	Pre-pregnancy Antihypertensive Medication Usage (Yes/No)			
8	**Current Blood Pressure** (mmHg)		Blood pressure taken at 6 months postpartum. Systolic blood pressure greater than 130 mmHg or diastolic blood pressure greater than 85 mmHg indicates increased risk.	
	Current Antihypertensive Medication Usage (Yes/No)			

The MOTHERS Program
The Mothers' Health Education, Research and Screening (MOTHERS) Program

History and Examination Continued

	Risk Indicator		Notes	Risk Factor
9	**Smoking** (Yes/No)		Smoking indicates increased risk.	
	If yes, number of cigarettes per day			
10	**Ever Smoked** (Yes/No)		A history of smoking indicates increased risk.	
	If yes, number of years smoked			
11	**Alcohol Consumption** (Yes/No)		Alcohol consumption may increase your risk.	
	If yes, number of drinks per week			
12	**Breastfeeding** (Yes/No)		Breastfeeding may affect a woman's ability to return to her pre-pregnancy weight.	
	If yes, duration in months			
13	**Physically Active** (Yes/No)		The federal guidelines of 30-60 minutes of moderate activity.	
	If active, number of times per week			
14	**Ethnicity**		South Asian, African and Metis/First Nations/Inuit are at increased risk.	
15	**Patient History of Major Cardiac Event** (Yes/No)		Patient history of MI or stroke indicates increased risk.	
16	**Patient History of Diabetes** (Yes/No)		Patient history of diabetes predating pregnancy indicates increased risk.	
17	**Patient History of Chronic Hypertension** (Yes/No)		Patient history of chronic hypertension indicates increased risk.	
18	**Family History of Hypertension/Preeclampsia in pregnancy** (Yes/No)		Any female family member on the maternal side with a self-reported history may indicate increased risk.	
19	**Family History of Hypertension** (Yes/No)		Family history of hypertension may indicate increased risk.	
20	**Family History of Major Cardiac Event** (Yes/No)		Family history of MI or stroke (<55 years of age in male relative and <65 years in a female relative may indicate increased risk).	
21	**Family History of Diabetes** (Yes/No)		Family history of diabetes (type 1, type 2 or gestational) may indicate increased risk.	
22	**Medications**	Some medications may affect a woman's risk of heart disease and/or may need to be taken into consideration when interpreting her biochemical test results. List current medications below.		
Total Number of Other Risk Factors				

Biochemical Testing

Biochemical Test	Result	Biochemical Test	Result
2 Hour 75g OGTT (Fasting) *Indicated only for women with a history of gestational diabetes.*	Fasting: ___ mmol/L 2 Hour: ___ mmol/L	**Total Cholesterol** (Fasting)	____ mmol/L
		HDL (Fasting)	____ mmol/L
HbA1C	_____ %	**LDL** (Fasting)	____ mmol/L
Glucose (Fasting)	____ mmol/L	**Triglycerides** (Fasting)	____ mmol/L
Urine Microalbumin: Creatinine	____ mg/mmol	**High Sensitivity CRP**	____ mg/L

Lifetime CVD estimate

Risk Factor	Stratification (Risk Level)	Patient's Risk Level (Please Circle)
Total Cholesterol (mmol/L)	<4.65 (optimal)	Optimal / Not Optimal / Elevated / Major
	4.65-5.15 (not optimal)	
	5.16-6.19 (elevated)	
	>6.20 (major)	
Systolic Blood Pressure (mmHg) *OR* **Currently Taking an Antihypertensive Medication**	<120 (optimal)	Optimal / Not Optimal / Elevated / Major
	120-139 (not optimal)	
	140-159 (elevated)	
	≥160 or Taking an Antihypertensive Medication (major)	
Diastolic Blood Pressure (mmHg) *OR* **Currently Taking an Antihypertensive Medication**	<80 (optimal)	Optimal / Not Optimal / Elevated / Major
	80-89 (not optimal)	
	90-99 (elevated)	
	≥100 or Taking an Antihypertensive Medication (major)	
Elevated Fasting Glucose (mmol/L) *OR* **Previous Diagnosis of Type 1 or 2 Diabetes**	≤6.88 (optimal)	Optimal / Major
	>6.88 or Diabetic (major)	
Smoking	No (optimal)	Optimal / Major
	Yes (major)	
Women's Lifetime CVD estimate (Lloyd-Jones et al., Circ 2006;113:791-798)		
• **All Optimal (8%)** • **≥1 Not Optimal (27%)** • **≥1 Elevated (39%)** • **1 Major (39%)** • **≥2 Major (50%)**		
Lifetime CVD Risk Estimate: ___ %		

Other Suggested Risk Calculations

(1) 30 Year CVD Risk Estimate

Your risk of developing cardiovascular disease at some point in the next 30 years is _____%.

(2) Metabolic Syndrome Calculation

Risk Factor	Scoring Cut Offs	Yes/No
Elevated Blood Pressure	≥ 130/85 mmHg	
Abdominal Obesity	> 88 cm waist circumference (>80 cm for Asian Ethnicity)	
Elevated Triglycerides	> 1.7 mmol/L	
Decreased HDL	< 1.3 mmol/L	
Elevated Fasting Glucose	> 5.6 mmol/L	
The metabolic syndrome criteria is met if 3 or more of the above risk factors are present.		

Appendix B. Research Ethics Certificates

B.1. Letter of Administrative Approval from the University of Ottawa Office of Research Ethics and Integrity.

24/08/2018

Université d'Ottawa
Bureau d'éthique et d'intégrité de la recherche

University of Ottawa
Office of Research Ethics and Integrity

Lettre d'approbation administrative I Letter of administrative approval

Numéro de dossier / Ethics File Number	H-08-18-1023
Titre du projet / Project Title	Identification of Molecular Subclasses of Preeclampsia
Type de projet / Project Type	Recherche de professeur / Professor's research project
CÉR primaire / Primary REB	Réseau de science de la santé d'Ottawa (RSSO) / Ottawa Health Science Network (OHSN)
Statut du projet / Project Status	Approuvé / Approved
Date d'approbation (jj/mm/aaaa) / Approval Date (dd/mm/yyyy)	24/08/2018
Date d'expiration (jj/mm/aaaa) / Expiry Date (dd/mm/yyyy)	18/10/2018

Équipe de recherche / Research Team

Chercheur / Researcher	Affiliation	Role
Shannon BAINBRIDGE-WHITESIDE	École interdisciplinaire des sciences de la santé / Interdisciplinary School of Health Sciences	Chercheur Principal / Principal Investigator
Samantha BENTON	Département de médecine cellulaire et moléculaire / Department of Cellular and Molecular Medicine	Co-chercheur / Co-investigator
Jeremiah GAUDET		Assistant de recherche / Research Assistant
Pascale ROBINEAU-CHARETTE	Département de médecine cellulaire et moléculaire / Department of Cellular and Molecular Medicine	Étudiant-chercheur / Student-researcher
Anika MUKHERJEE	École interdisciplinaire des sciences de la santé / Interdisciplinary School of Health Sciences	Étudiant-chercheur / Student-researcher

Conditions spéciales ou commentaires / Special conditions or comments:

B.2. Certificate of Ethics Approval from the University of Ottawa Office of Research Ethics and Integrity.

30/03/2022

Université d'Ottawa
Bureau d'éthique et d'intégrité de la recherche

University of Ottawa
Office of Research Ethics and Integrity

CERTIFICAT D'APPROBATION ÉTHIQUE I CERTIFICATE OF ETHICS APPROVAL

Numéro du dossier / Ethics File Number	H-08-18-1023
Titre du projet / Project Title	Identification of Molecular Subclasses of Preeclampsia
Type de projet / Project Type	Recherche de professeur / Professor's research project
Statut du projet / Project Status	Renouvelé / Renewed
Date d'approbation (jj/mm/aaaa) / Approval Date (dd/mm/yyyy)	24/08/2018
Date d'expiration (jj/mm/aaaa) / Expiry Date (dd/mm/yyyy)	27/10/2022

Équipe de recherche / Research Team

Chercheur / Researcher	Affiliation	Role
Shannon BAINBRIDGE-WHITESIDE	École interdisciplinaire des sciences de la santé / Interdisciplinary School of Health Sciences	Chercheur Principal / Principal Investigator
Anika MUKHERJEE	École interdisciplinaire des sciences de la santé / Interdisciplinary School of Health Sciences	Étudiant-chercheur / Student-researcher
Dina EL DEMELLAWY	university of Ottawa	Co-chercheur / Co-investigator
Purvasha PATNAIK	École interdisciplinaire des sciences de la santé / Interdisciplinary School of Health Sciences	Étudiant-chercheur / Student-researcher
Erika MERY	École interdisciplinaire des sciences de la santé / Interdisciplinary School of Health Sciences	Étudiant-chercheur / Student-researcher

Conditions spéciales ou commentaires / Special conditions or comments

B.3. Certificate of Completion of TCPS-2: CORE Course for Erika Mery

PANEL ON RESEARCH ETHICS

Navigating the ethics of human research

TCPS 2: CORE

Certificate of Completion

This document certifies that

Erika Mery

has completed the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans Course on Research Ethics (TCPS 2: CORE)

Date of Issue: **9 July, 2018**

Appendix C. Scholarly Achievements

C.1. Scholarships

University of Ottawa Graduate Admission Scholarship (September 2020-August 2022)

Canada Graduate Scholarship- Master's Award (May 2021-May 2022)

C.2. Publications

Benton SJ, Mery EE, Grynspan D, Gaudet LM, Smith GN, Bainbridge SA. Placental Pathology as a Tool to Identify Women for Postpartum Cardiovascular Risk Screening following Preeclampsia: A Preliminary Investigation. Journal of Clinical Medicine. March 2022;11(6).

Dancey S, Mery E, Esteves A, Oltean I, Hayawi L, Tang K, et al. Placenta pathology in recipient versus donor oocyte derivation for in vitro fertilization in a setting of hypertensive disorders of pregnancy and IUGR. Placenta. May 2021;108:114–21.

C.3. Published Abstracts

Mery E, Dancey S, Esteves A, Oltean I, Hayawu L, Tang K, et al. Histopathology findings in pregnancies with placental disease according to recipient versus donor oocyte derivation for in vitro fertilization. Placenta. 2021 Sep;112:e43–4.

Mery E, Benton S, Smith G, Grynspan D, Bainbridge S. The use of placenta pathology to identify women at high-risk of cardiovascular disease following preeclampsia. Canadian Journal of Cardiology. 2021 Feb;37(2):e3.

C.5. Presentations

1. The use of placenta pathology to identify women at high-risk of cardiovascular disease following preeclampsia. Poster presentation at the University of Ottawa Faculty of Health Science Research Day, 2022.

2. The use of placenta pathology to identify women at high-risk of cardiovascular disease following preeclampsia. Oral presentation at the Canadian Women's Heart Summit, 2021.
3. Histopathology findings in pregnancies with placental disease according to recipient versus donor oocyte derivation for in vitro fertilization. E-poster presentation at the International Federation of Placenta Associations Annual Meeting, 2021.
4. Histopathology findings in pregnancies with placental disease according to recipient versus donor oocyte derivation for in vitro fertilization. Oral presentation at the Children's Hospital of Eastern Ontario Research Day, 2020.

Appendix D. Permission for Publications

D.1. Permission to include *Placental pathology as a tool to identify women for postpartum cardiovascular risk screening following preeclampsia: a preliminary investigation* in a thesis