Association Between Cardiometabolic Pregnancy Complications and Cardiovascular Diseases

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March 2017

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Master in Epidemiology and Biostatistics

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ABSTRACT

Background: Cardiometabolic pregnancy complications including hypertensive disorders of pregnancy (HDP) and gestational diabetes (GDM) each double the risk of cardiovascular disease (CVD) later in life. Two hypotheses have been posited to explain this increase in risk: shared burden of atherosclerotic risk factors, and persistent vascular impairment after the affected pregnancy. Thus, the aim of this work is to explore the biological mechanism underlying the association between pregnancy complications and CVD as well as the clinical outcomes at the time of an ischemic event.

Methods: 1. Through a systematic review and meta-analysis, we summarized and updated evidence for sustained vascular dysfunction at least three months after HDP, as measured by imaging modalities and serum biomarkers. We pooled results of modalities reported in more than three studies using a random effects model. 2. Using data from the GENESIS-PRAXY prospective cohort, we studied 251 parous women (≤ 55 years old) hospitalized with an acute coronary syndrome (ACS) in whom detailed medical and obstetric history as well as biological data were available. We compared clinical presentation, traditional risk factors and biomarkers of endothelial dysfunction at ACS diagnosis in women with versus without a prior history of complicated pregnancy (HDP and/or GDM). Major adverse cardiac events (MACE) were captured at 12 months.

Results: 1. By summarizing more than 70 studies, we found evidence of sustained vascular dysfunction after pregnancies complicated with HDP. There was evidence of vascular dysfunction in women post HDP compared to women with prior normal pregnancy when measured by carotid-femoral pulse-wave velocity (0.64m/s [0.17 to 1.11]), carotid intima-media thickness (0.025mm [0.004 to 0.045]) and augmentation index (5.48% [1.58 to 9.37]), as well as mean levels of soluble fms-like tyrosine kinase (6.12pg/ml [1.91 to 10.33]). Vascular dysfunction was more pronounced in younger women (< 40 years) and closer to the index pregnancy. 2. At the time of the ACS, women with previous pregnancy complications were younger (47.4 ± 6.2 vs. 49.1 ± 5.6 years, p=0.002), and had a greater burden of traditional atherosclerotic risk factors compared with women with prior normal pregnancy. Of note, women with prior preeclampsia were more likely to have chronic hypertension and to present with ST-elevation myocardial infarction as compared to women with prior unaffected pregnancy (adjusted OR 2.76 [1.04, 7.29]). At 12 months, there was a trend for increased risk of MACE in women with prior

pregnancy complications, mostly driven by an increased risk of recurrent ACS in women with prior preeclampsia.

Conclusion: Pooled data from studies evaluating vascular imaging suggest that some vascular dysfunction persists in women with prior HDP as compared to women with prior normal pregnancy. Women with prior pregnancy complications also present with ACS at a younger age, and with a high burden of atherosclerotic risk factors. In particular, preeclampsia was associated with more severe ACS at presentation and a higher likelihood of recurrence. Further studies are needed to understand the precise trajectory between cardiometabolic pregnancy complications and development of CVD, and the development and testing of tailored interventions.

RÉSUMÉ

Mise en contexte: Les complications cardio-métaboliques de grossesse, incluant les troubles hypertensifs de la grossesse et le diabète gestationnel, doublent le risque de maladie cardiovasculaire (MCAS) maternelle à long terme. Deux hypothèses ont été proposées pour expliquer l'augmentation du risque: un profil similaire de facteurs de risque athérosclérotique et la persistance d'un dommage vasculaire après la grossesse. Ainsi, l'objectif de ce travail est d'explorer les mécanismes biologiques soutenant l'association entre les complications de grossesse et la MCAS ainsi que les issues cliniques au moment d'un évènement ischémique.

Méthode : 1. À l'aide d'une revue systématique et d'une méta-analyse, nous avons résumé et actualisé les évidences d'un dommage vasculaire persistant mesuré au minimum trois mois après un trouble hypertensif de la grossesse, et ce par des techniques d'imagerie et des biomarqueurs sanguins. À l'aide d'un modèle à effet aléatoire, nous avons regroupé les résultats de chaque modalité lorsque ceux-ci étaient rapportés par plus de trois études. 2. À l'aide des données de la cohorte prospective GENESIS-PRAXY, nous avons étudié 251 femmes (≤ 55 ans) hospitalisées pour un syndrome coronarien aigu (SCA) ayant déjà eu une grossesse et dont les renseignements médicaux, obstétricaux et biologiques étaient disponibles. Nous avons comparé les présentations cliniques, les facteurs de risque athérosclérotique et les biomarqueurs de dysfonction endothéliale au moment du diagnostic du SCA entre les femmes ayant eu ou non un antécédent de complication cardio-métabolique de grossesse. Les évènements cardiaques majeurs (MACE) 12 mois post-SCA ont été recensés.

Résultats : 1. En résumant plus de 70 études, nous avons démontré la persistance d'un dommage vasculaire chez les femmes ayant eu un trouble hypertensif de la grossesse comparativement à celles avec grossesses antérieures normales à l'aide de l'étude fémoro-carotidienne des ondes pulsatiles (0.64m/s [0.17, 1.11]), l'épaisseur de l'intima-média carotidien (0.025mm [0.004, 0.045]), l'index d'augmentation vasculaire (5.48% [1.58, 9.37]), ainsi qu'avec les niveaux moyens du soluble fms-like tyrosine kinase (6.12pg/ml [1.91, 10.33]). Les données ont démontré que le dommage vasculaire était plus prononcé chez les jeunes femmes (<40 ans) et aussi lorsque mesuré plus tôt après la grossesse. 2. Au moment du SCA, les femmes ayant eu des complications de grossesse étaient plus jeunes (47.4 ± 6.2 vs. 49.1 ± 5.6 ans, p=0.002) et avaient plus de facteurs de risque athérosclérotique comparativement aux femmes avec grossesses antérieures normales. Les femmes avec une pré-éclampsie antérieure souffraient plus d'hypertension chronique et se présentaient plus souvent avec un infarctus du myocarde avec

élévation du segment ST comparativement aux femmes avec des grossesses antérieures normales (rapport de cote ajusté 2.76 [1.04, 7.29]). Douze mois post-SCA, les femmes ayant eu des complications de grossesse avaient plus tendance à souffrir d'un MACE, ce qui était surtout le reflet d'une augmentation du risque de récidive d'un SCA chez les femmes avec antécédent de pré-éclampsie.

Conclusion : Les données regroupées provenant d'études évaluant des techniques d'imagerie suggèrent la persistance d'un dommage vasculaire chez les femmes ayant eu des troubles hypertensifs de la grossesse comparativement aux femmes avec grossesses antérieures normales. De plus, les femmes ayant eu une complication de grossesse sont plus jeunes au moment du SCA et ont plus de facteurs de risque athérosclérotique. En particulier, les femmes ayant eu une pré-éclampsie se présentent avec des SCA plus sévères et ont un plus grand risque de récurrence à 12 mois. Des études subséquentes sont nécessaires afin de bien comprendre le mécanisme précis entre les complications cardio-métaboliques de grossesse et le développement subséquent de MCAS, ainsi que le développement de stratégies de prévention à être testées.

ACKNOWLEDGEMENTS

I am very grateful to Dr. Louise Pilote for her supervision of my Master's thesis and her continuous support throughout this adventure. She helped me consolidate my epidemiological knowledge acquired through my Master's courses. Her leadership with her research team has inspired me and will continue to influence my own research career.

I am also indebted to Dr. Natalie Dayan for her continuous counsel during my Master's journey and for her clinical expertise. The constructive discussions we had together helped me to acquire confidence in my work. Her mentorship has truly enriched my research experience over the past two years.

I would also like to thank the GENESIS-PRAXY team for their support. A special thank to Jasmine Poole, an extraordinary research coordinator, who has solutions for all problems whether they are about research or personal life. Another special thank to Marisa Okano who helped and supported me in the long process of my systematic review and throughout my thesis's writing. I have also enjoyed the support of two mentors: Dr. Roxanne Pelletier and Dr. Amanda Rossi. I also thank Dr. Hassan Behlouli for his statistical expertise and assistance throughout my studies.

Financial support for my Master's thesis was provided by the Canadian Vascular Network and McGill University.

On a personal note, I would like to thank my family and friends for their support over the past two years. Coming back to school after five years of general internal medicine residency was not always easy but they encouraged me during all the process. I would not have achieved this without them. A special thank to Patato.

PREFACE & CONTRIBUTIONS OF AUTHORS

This thesis was written following the manuscript-based thesis guidelines. I am the first author of the two manuscripts that present the substantive contribution of my work. I performed the literature review, established the methods and design, analyzed and interpreted the data, and wrote the manuscripts. I also presented these two manuscripts in national and international conferences and prepared the submissions to peer-reviewed journals.

Drs. Pilote and Dayan helped with the study design, statistical analyses, interpretation of data, and critical revision and editing of the manuscripts. For the first manuscript, Tara Landry helped with the development of the search strategy and literature review, while Marisa Okano was the second reviewer for the selection process and data extraction. They also both gave critical revision of the manuscript. For the second manuscript, Marisa Okano helped in the development of our cohort of pregnant women and organized blood samples shipping to Kenny Schlosser who did the investigational biomarkers analysis. Duncan J Stewart supervised the work of Kenny Schlosser. The three of them gave critical revision of the manuscript. Dr Pilote acquired the funding for all studies.

INTRODUCTION

Over the past decade, the rate of hospitalizations for acute coronary syndrome (ACS) has been stable in men below 55 years old, but it has increased among women.[1] Women with ACS have also a higher mortality rate during hospitalization[2] and up to 2 years after the event.[3] Results from a recent 10-year cohort indicated that the overall odds of early mortality in women under 55 years old hospitalized for ACS was 45% higher than men of the same age.[4] It has been shown that young women (≤ 55 years old) with a family history of cardiovascular disease (CVD) have the greatest burden of traditional atherosclerotic risk factors at the time of ACS.[5] However, the presence of traditional CVD risk factors alone do not account for this disparity.[4,6,7] The explanation for this disparity is likely multifactorial, and likely includes genetic, psychosocial, and sex-specific factors. The hypothesis that some sex-specific risk factors may have an influence on women's higher cardiovascular risk was raised a few years ago.

There are several known female-specific vascular risk factors including oral contraceptives, hysterectomy, menopause, and hormone replacement therapy.[8] Perhaps the most important and increasingly recognized sex-specific CVD risk factors are pregnancy complications. Mann et al. were the first to report that the preeclamptic state "toxaemia" was an independent risk factor for future myocardial infarction.[9] Since then, many studies have assessed the association between preeclampsia, as well as other placental and metabolic pregnancy complications, and the future development of CVD.[10] The underlying biological explanation for this observed association is not clear, and may reflect (i) shared burden of traditional atherosclerotic risk factors for pregnancy complications and frank CVD; and/or (ii) vascular dysfunction resulting from severe placental complications during pregnancy, causing accelerated vascular aging and leading to downstream CVD.

The overall aim of this work is to explore biological mechanisms underlying the association between pregnancy complications and CVD in women and its consequences at the time of an acute ischemic event.

BACKGROUND

1.0 Physiologic Changes During a Normal Pregnancy

Pregnancy is akin to a first cardiac stress-test in a woman's life.[11] Pregnancy is a pro-atherogenic metabolic state due to insulin resistance,[12] hypercoagulability,[13] high cardiac output,[14] increased inflammatory activity,[15] and hyperlipidemia.[16] Indeed, during a normal pregnancy, fasting plasma insulin is almost twice as high in late pregnancy as post-partum;[17] the decrease in blood glucose after an insulin infusion is lower[18] and there is a decrease in glucose utilization rates.[19] Moreover, higher levels of procoagulant factors[20] combined with impaired fibrinolysis[13] increase the risk of thrombosis. There is also a 35-50% increase in cardiac output accompanied by an increase in heart rate and of approximately 40% of the plasma volume.[21] All these changes occur to meet the increase metabolic demands of the mother and the growing foetus. In pregnancy, there is a progressive increase in triglycerides and cholesterol concentrations in the maternal plasma.[22] In the late second trimester, there is also a release of free fatty acids and an increase in the hepatic output of very low-density lipoproteins (VLDL), which are atherogenic.[23] The great majority of women tolerate these metabolic alterations but in some cases, these changes are either exaggerated or poorly tolerated leading to specific conditions during pregnancy such as gestational diabetes mellitus (GDM) and hypertensive disorders of pregnancy (HDP).[24]

2.0 Metabolic and Hypertensive Complications of Pregnancy

2.1 Pathogenesis and Epidemiology of Gestational Diabetes Mellitus

GDM is defined as an increase in blood glucose level occurring for the first time during pregnancy.[25] As explained previously, the pregnancy state is associated with an increase insulin resistance due to placental secretion of diabetogenic hormones including growth hormone, corticotropin-releasing hormone from which is derived cortisol, human placental lactogen hormone and progesterone.[26] These and other metabolic changes take place to provide sufficient nutrients to the foetus. GDM occurs when a woman's pancreatic β-cells are insufficient to increase insulin secretion to overcome this insulin resistance and this phenomenon is more likely to occur in women with pre-existing insulin resistance.[24]

The definition of GDM is a moving target. The Hyperglycaemia and Adverse Pregnancy Outcomes[27] study evaluated the impact of the entire spectrum of hyperglycaemia in pregnancy. In over 23 000 pregnant women, the authors found a linear continuous association between maternal glucose levels during pregnancy and the primary outcomes (macrosomia, clinical neonatal hypoglycaemia, primary caesarean section and cord-blood serum C-peptide level).[27] Following these findings, the International Association of the Diabetes and Pregnancy Study Group (IADPSG) has since recommended universal screening with a 75g oral glucose tolerance test between 24 and 28 weeks of gestation and has recommended using lower glycemic thresholds to diagnose the disorder than previously published guidelines.[28] These guidelines have been approved by the World Health Organisation (WHO) and partially by the Canadian Association of Diabetes but not by all international associations, and have effectively increased the prevalence of GDM [29,30,31]

The prevalence of GDM, which is now reported to be between 2 to 10%, depends on the current accepted definition of the disorder, and could increase to almost 20% if all worldwide associations would follow IADPSG recommendations. Such screening would also increase the cost of medical care for pregnant women.[31] However, guidelines proponents argue that increase in prevalence would rather be a reflection of the increasing prevalence of pre-diabetes in women of childbearing age in the past years,[32] as undiagnosed type II diabetes is often misdiagnosed as GDM in pregnancy. The increasing maternal age at delivery in the past years, which is a strong risk factor for GDM, could also be a culprit.[32] The other known risk factors are previous GDM, pre-diabetes, ethnicity, body mass index (BMI) $\geq$ 30 kg/m^2, polycystic ovarian syndrome, corticosteroid use, previous macrosomic infant ($\geq$ 4.5 kg) and current fetal macrosomia or polyhydramnios.[29]

2.2 Hypertensive Disorders of Pregnancy

2.2.1 Classification and Definitions

HDP comprise closely related syndromes: gestational hypertension with or without superimposed preeclampsia/eclampsia, de novo preeclampsia/eclampsia, and chronic hypertension with or without superimposed preeclampsia/eclampsia.[33,34] These disorders can occur antepartum or in the immediate post-partum period and usually resolve by 12 weeks post-partum.

HDP affect about 5 to 10% of pregnancies.[35] The incidence of preeclampsia ranges from 3 to 5% but may increase to 25% in women with pre-existing hypertension.[36] In contrast, the prevalence of gestational hypertension is estimated at 6 to 7%.[21] HDP remain the leading cause of maternal and perinatal morbidity and mortality especially when severe preeclampsia occurs.[34] Preeclampsia is one of the most common cause of prematurity and accounts for 25% of all infants with very low birth weight (< 1500 g).[37]

The major risk factors for preeclampsia can be divided into different categories: demographics and family history (maternal age over 40, familial history of preeclampsia or early-onset of CVD), past medical or obstetric history (anti-phospholipid antibody syndrome, pre-existing medical conditions as chronic hypertension, diabetes mellitus or renal disease, previous preeclampsia), and factors about the current pregnancy (multiple gestation, high BMI before conception, reproductive technologies).[34]

In 2014, the Society of Obstetricians and Gynaecologists of Canada (SOGC) published the most recent clinical guidelines on diagnosis, evaluation and management of HDP.[34] In these guidelines, hypertension is defined as blood pressure ≥ 140/90 mmHg measured in the office setting on at least two measures.[38] Chronic hypertension implies hypertension that is present before pregnancy or that is diagnosed before 20 weeks of gestation, while gestational hypertension is diagnosed after 20 weeks of gestation. Preeclampsia may either complicate chronic and gestational hypertension or may arise de novo.

2.2.2 Pathogenesis of Preeclampsia

The pathogenesis of preeclampsia is not well understood. One commonly proposed model stipulates that the placenta is the principal involved organ. In this model, not even the foetus is required for the development of preeclampsia as it also occurs in molar pregnancies.[39] In normal pregnancy, there is a remodelling of spiral arteries (which perfuse the placenta) induced by trophoblastic cells. This remodelling results in modification of the endothelium and allows dilatation of the blood vessels [10] to improve exchanges between the mother and the foetus.[40] The hallmark of preeclampsia is a failure in this remodelling, otherwise called “poor placentation”, which results in narrower blood vessels and hypoperfusion of the placenta.[41] Within this hypoxic environment ensues the release of inflammatory cytokines and antiangiogenic proteins and consequent widespread endothelial dysfunction.[39,40] This dysfunction creates an imbalance of

circulating pro- and antiangiogenic proteins, which further reduces perfusion of the placenta by vasoconstriction.[39] Perfusion is also compromised by the activation of the coagulation cascade, especially platelets, which creates a pro-thrombotic state and micro-thrombi.[42] Consequent decreased organ perfusion gives rise to the classical symptoms of preeclampsia as it is a systemic vascular disorder:[39] hypertension, proteinuria, oedema, headache, visual scotoma, reduced glomerular filtration and foetal growth restriction.[43]

3.0 Long-Term Cardiovascular Risk After Pregnancy Complications

3.1 Following Gestational Diabetes Mellitus

Resolution of GDM usually occurs soon after delivery of the placenta. Canadian guidelines recommend screening for hyperglycemia between 6 weeks and 6 months post-partum because of the known association between GDM and frank diabetes mellitus.[44] A population-based study in Ontario had shown that 3.7% of women with GDM are diagnosed with type II diabetes as early as 9 months post-partum. This rate increases to 18.9% by 9 years in comparison to 2% among women without GDM.[45]

Perhaps independent of the strong risk for type II diabetes mellitus, women with previous GDM are also at risk of CVD later in life. Several large observational studies have reported hazard ratios (HR) ranging from 1.13 (95% confidence interval [CI] 0.67 to 1.89) to 1.71 (95% CI 1.08 to 2.9) 10-15 years following the index pregnancy.[46,47] While some of these effects are after adjustment for type II diabetes, in other studies the apparent association was attenuated after adjustment for diabetes mellitus.

3.2 Following Hypertensive Disorders of Pregnancy

It was previously believed that HDP resolve after delivery of the placenta leaving no long-term sequelae for the mother. In 1950, Chesley et al reported that toxaemia of pregnancy (former name for preeclampsia) could have hypertensive sequelae many years after the index pregnancy[48] and Mann et al, few years later, made the link with CVD.[9] Since then, many observational studies have demonstrated a link between HDP and long-term CVD.[49,50] The first meta-analysis on the subject was published in 2007.[51] It included eight studies comprising 2 346 997 women and showed a pooled relative risk (RR) of 2.16 (95% CI 1.86 to 2.52) of ischemic heart disease in women with a previous history of preeclampsia compared to women with previous normal

pregnancy at a mean of 11.7 years after the index pregnancy. The study with the shortest duration of follow-up (7.8 years) also found a RR of 2.2 (95% CI 1.3 to 3.6) of CVD after preeclampsia.[52] The magnitude of this risk has been associated with the severity of preeclampsia, the gestational age at onset and recurrent preeclampsia.[53] Severe preeclampsia, which developed before 34 weeks of gestation and is complicated by pre-term birth, has the highest association with CVD and related deaths.[49] In 2013, an updated systematic review and meta-analysis including 18 articles was published in the European Journal of Epidemiology and presented similar conclusions; the odds ratio (OR) of CVD later in life was 2.28 (95% CI 1.87 to 2.77) for women who experienced preeclampsia.[54]

While there appears to be a strong and consistent association between GDM and HDP with premature CVD, there are a number of unanswered questions. Owing to challenges in prospectively following large groups of women after pregnancy until the development of CVD, it is unclear what is the exact mechanism behind each of these associations.

3.3 Major Hypotheses

Two major hypotheses are proposed to explain the link between previous pregnancy complications and CVD. The first stipulates that HDP, GDM and CVD share the same burden of risk factors (obesity, hypertension, diabetes and advanced age). Thus, pregnancy complications could be the first expression of a cardiovascular phenotype.[53,55] The second hypothesis implicates vascular impairment induced mostly by preeclampsia, which creates permanent damage to the endothelium, induces chronic hypertension, accelerates atherosclerosis and increases the risk of CVD.[10] Even if these two hypotheses are not mutually exclusive, their relative role in explaining ACS in women with previous pregnancy complications is poorly understood.

3.3.1 Cardiovascular Phenotype

For many years, HDP have been linked to chronic hypertension after the index pregnancy such as the link between GDM with type II diabetes.[51,56] A meta-analysis including 21 030 women, with a mean duration of follow-up of 14.1 years (5 to 32.65), showed that the RR of developing chronic hypertension after preeclampsia was 3.70 (95% CI 2.7 to 5.0), while it was

3.39 (95% CI 0.82 to 13.92) for women with gestational hypertension, both compared to women with previous normal pregnancy.[51] The same group published a meta-analysis on the RR of developing type II diabetes after GDM and found it to be 7.43 (95% CI 4.79 to 11.51).[57]

These two pregnancy complications are also interrelated. HDP have been associated with a higher insulin resistance in early pregnancy independently of obesity and glucose tolerance.[58,59] This higher insulin resistance in women who develop preeclampsia suggests an association between HDP and type II diabetes later in life. A population-based cohort study of 16.5 years follow-up with administrative health claims in Ontario (1 010 068 women) showed that gestational hypertension was associated with a HR of 1.95 (95%CI 1.83 to 2.07) of diabetes later in life, while preeclampsia had a HR of 2.08 (95%CI 1.97 to 2.19).[60] This HR increases to 15.75 (95% CI 14.25 to 17.07) when preeclampsia was associated with GDM. Women with GDM are also at risk of chronic hypertension later in life.[61] Moreover, these two pregnancy complications have also been linked to the other components of the metabolic syndrome.[46,62,63]

An important question is if these risk factors were already present before the index pregnancy. The CHAMPS study (Cardiovascular Health After Maternal Placental Syndrome) linked health care administrative databases in Ontario and included 1 033 559 women of which 7% had a maternal placental syndrome (preeclampsia 49%, gestational hypertension 28%, placental abruption 15% and placental infarction 12%).[64] The authors looked at the diagnosis of traditional atherosclerotic risk factors within 24 months before the index delivery. Women with maternal placental syndrome were more likely to have pre-pregnancy hypertension, diabetes, dyslipidemia or obesity. Moreover, women who had maternal placental syndrome and one or two traditional risk factors had HR for future CVD of 4.5 (95% CI 3.7 to 5.4), while the HR for women with three of four risk factors was 11.7 (95% CI 4.9 to 28.4), both compared to women who had neither.

However, even if we conclude that women with pregnancy complications are at higher risk to previously have or to develop traditional risk factors after the index pregnancy, we still do not know if at the time of ACS these women have a more important burden of risk factors compared with women without pregnancy complications or if their increased risk is mediated through some other mechanism.

3.3.2 Vascular Impairment

Persistent vascular impairment includes endothelial dysfunction, arterial stiffness, and subclinical atherosclerosis**.** Endothelial dysfunction is a systemic disorder arising from the inner lining of blood vessels that leads to abnormal vascular tonoregulation and aggregation of platelet and monocytes. Both of these processes are involved in the pathophysiology of atherosclerosis.[65] Endothelial dysfunction is strongly related to clinically apparent CVD, in particular in populations lacking traditional CVD risk factors.[66] It can be measured in a number of ways including imaging modalities and serum biomarkers. Flow mediated dilatation (FMD) is the gold standard test to measure endothelial dysfunction. Low FMD is a predictor of an increase incidence of CVD at 5 years.[67]

Arterial stiffness is strongly associated with atherosclerosis and is an indirect sign of global endothelial dysfunction and vascular impairment.[68] Pulse wave velocity (PWV) is the gold standard method to assess arterial stiffness and has been independently associated with an increased risk of vascular disease.[69] A recent meta-analysis demonstrated that an increase in PWV by 1 m/s corresponds to an adjusted risk increase of 14%, 15% and 15% in total vascular disease events, vascular disease mortality, and all-cause mortality, respectively.[70]

Common carotid artery intima-media thickness (cIMT) is a marker of subclinical atherosclerosis and is also a valid surrogate of vascular morbidity and mortality risk.[71] Increased cIMT has been shown to predict the risk of CVD in the general population including type II diabetes.[72] It can also improve global cardiovascular risk prediction in addition to hypertension.[73]

There have been several attempts to measure vascular impairment using serum biomarkers. These biomarkers have been studied in comparison to imaging modalities (FMD, PWV, cIMT) or in people known to have CVD. Cellular adhesion molecules (CAMs), which include soluble vascular CAM-1 (sVCAM-1) and intercellular CAM-1 (sICAM-1), play a critical role in the pathophysiology of atherosclerosis by mediating the adhesion and migration of leukocytes.[74] Rohde et al demonstrated a positive association between serum CAMs and cIMT after adjustment for traditional CVD risk factors.[74] Another important biomarker of CVD is soluble endoglin (sEng). sEng plays an important role in vascular function and has profibrotic effect.[75] It has been associated with atherosclerosis plaque vulnerability[76] and is increased in ACS.[77] The most novel biomarkers in this field are microRNAs (miRNAs), which represent more than 2500 short (20-25 base pairs) RNA sequences that regulate gene expression.[75] They have

been linked to many diseases including CVD, such as myocardial hypertrophy, infarction, angiogenesis, fibrosis and heart failure.[78,79] In the past years, they have emerged as one of the most important therapeutic target in CVD.[80]

3.3.2.1 Vascular Impairment and Pregnancy Complications

GDM and HDP each result in widespread endothelial activation and dysfunction during pregnancy, through different mechanisms.[72,81] In GDM, the hyperglycaemic state is the primary culprit resulting in endothelial dysfunction.[72] Hyperglycaemia creates it via the liberation of free radicals directly from glucose auto-oxidation[82] or indirectly from the prostaglandins secreted during a hyperglycaemic state.[83] These free radicals inactivate endothelium-derived nitric oxide and interfere with endothelium vasodilatation.[84]

As described previously in section 2.2.2, endothelial dysfunction has also a central role in the pathogenesis of preeclampsia, and likely explains how this disorder affects many different maternal organs. By its pathophysiology, preeclampsia causes endothelial dysfunction by the release of inflammatory cytokines in response to hypoperfusion of the placenta.[24]

Different serum biomarkers of vascular impairment associated with preeclampsia have been studied in the past years. Level of soluble fms-like tyrosine kinase 1 (sFlt-1), a circulating antiangiogenic protein, increases during preeclampsia. It causes endothelial dysfunction by reducing circulating level of vascular endothelial growth factor (VEGF) and placental growth factor (PIGF),[85] preventing their interaction with endothelial receptors on cell surface.[86] An elevated ratio of sFlt-1 to PIGF has been found to be predictive of preeclampsia.[87] Many other biomarkers were looked at during preeclampsia but very few of them have a prognosis utility.[88]

Moreover, whether or not vascular impairment persists after pregnancy complications is still subject of discussion. Results from studies that looked at imaging modalities (e.g. PWV, cIMT and FMD) are conflicting. Some of them found a difference between women with pregnancy complications and those without, more than ten years after the index pregnancy, while others did not.[89-91] Results are also contradictory for the persistence of serum biomarkers of vascular impairment after the index pregnancy between women with and without prior pregnancy complications.[92,93]

These two hypotheses (shared burden of traditional atherosclerotic risk factors and persistent vascular dysfunction) have been proposed by experts, since many years now. However, until now, no clear mechanism has been established and many knowledge gaps remain.

SUMMARY AND RATIONALE

The rate of hospitalizations for ACS is higher in young women than in men of the same age and this disparity could not fully be explained by a higher prevalence of traditional atherosclerotic risk factors. This disparity is likely multifactorial including sex-specific risk factors as cardiometabolic pregnancy complications. Pregnancy is a pro-atherogenic metabolic state[12-16] and is now considered as the first cardiac stress-test in a woman's life.[11] Pregnancy complications, including GDM and HDP, are important established biological risk factors for future CVD in women.[51,57] Two major hypotheses are suggested as mechanisms for this association: shared burden of risk factors known as the cardiovascular phenotype,[53,55] and the persistence of vascular dysfunction after pregnancy that accelerates atherosclerosis.[10] Many studies have been looking at these two hypotheses, but there remains uncertainty as to which of the two hypotheses is most plausible or how they are interlinked. Moreover, even if the increase risk of CVD after pregnancy complications has been established, the impact of those pregnancy complications on the clinical presentation, severity and outcomes of a cardiovascular event is still unknown.

OBJECTIVES AND HYPOTHESES

The overall objective is to explore biological mechanisms underlying the association between pregnancy complications and CVD in women.

Specific Objectives:

1. To systematically review and summarize the evidence for sustained vascular impairment measured by direct serum biomarkers or imaging modalities following HDP.

2. To estimate associations between pregnancy complications and traditional CVD risk factors and biomarkers of endothelial dysfunction in women at the time of premature ACS.

3. To evaluate the impact of pregnancy complications on clinical severity and Major Adverse Cardiac Events (MACE) up to 12 months following premature ACS.

Hypotheses

1. Vascular impairment persists after the index pregnancy and is likely implicated in the increased risk of CVD in women who have had a HDP.

2. The burden of traditional risk factors at the time of ACS will be higher in women with prior pregnancy complications as compared with women without these complications, reflecting longstanding metabolic dysfunction following a complicated pregnancy. Biomarkers of vascular dysfunction will be found in a higher proportion at the time of ACS in women with prior HDP, indicating the importance of vascular dysfunction in the association between HDP and CVD.

3. Women with prior pregnancy complications have had poor vascular health for a longer period of time compared with women with prior normal pregnancy, predisposing them to more severe ACS and worse outcomes following ACS.

PREFACE TO MANUSCRIPT #1

. Persistent vascular dysfunction following HDP, including endothelial dysfunction, arterial stiffness and subclinical atherosclerosis, has been suggested as a putative mechanism for the increased long-term risk of CVD. Several vascular imaging modalities assessing various types of vascular dysfunction have been studied post-partum: endothelial dysfunction by FMD, forearm blood flow, laser doppler and endoPAT, arterial stiffness by PWV and augmentation index, and subclinical atherosclerosis by cIMT. Some serum biomarkers directly linked to vascular dysfunction either alone or in association with preeclampsia have also been studied, including markers of angiogenesis (sFlt-1, VEGF, PIGF, sENG), inflammation (sICAM-1, sVCAM-1), and thrombosis (endothelin, and fibronectin). However, results have been inconsistent and it remains unclear if some degree of vascular dysfunction persists in women following pregnancies complicated by HDP. The objective of this study was to summarize and update evidence for sustained vascular dysfunction at least three months following HDP, as measured by imaging modalities and serum biomarkers.

This manuscript was published in Hypertension in September 2016. An abstract, based on the results of this study, was presented as a moderated poster at the American College of Cardiology Annual Meeting in Chicago and as an oral presentation at the Canadian Women's Heart Health Summit in Ottawa, both in April 2016. To our knowledge, this is the first meta-analysis to include imaging modalities and serum biomarkers to assess the persistence of vascular impairment after HDP.

MANUSCRIPT #1

MARKERS OF VASCULAR DYSFUNCTION AFTER HYPERTENSIVE DISORDERS OF PREGNANCY: A SYSTEMATIC REVIEW AND META-ANALYSIS

Short title : Pregnancy Complications And Vascular Dysfunction

Manuscript number: HYPE201607907

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Word count of manuscript: 7269

Word count of abstract: 249

Total number of figures: 3

ABSTRACT

Women with prior hypertensive disorders of pregnancy are at twice the risk of cardiovascular disease compared to women with prior normotensive pregnancy, possibly due to sustained vascular dysfunction following delivery. The aim of this systematic review and meta-analysis is to summarize evidence of vascular dysfunction at least three months following hypertensive disorders of pregnancy. Articles in all languages were retrieved from principal databases. Studies included were observational with hypertensive disorders of pregnancy as the main exposure and measurements of vascular dysfunction via imaging modalities or serum biomarkers as the main outcome, assessed at least 3 months postpartum. We pooled results of modalities reported in more than three studies using a random effects model. Of 6109 potentially relevant studies, 72 were included that evaluated 10 imaging modalities and 11 serum biomarkers in 8702 women. There was evidence of vascular dysfunction in women post-hypertensive disorders of pregnancy compared to women with prior normal pregnancy when measured by carotid-femoral pulse-wave velocity (0·64m/s [0·17 to 1·11]), carotid intima-media thickness (0·025mm [0·004 to 0·045]) and augmentation index (5·48% [1·58 to 9·37]), as well as mean levels of soluble fms-like tyrosine kinase (6·12pg/ml [1·91 to 10·33]). Between groups differences in measures of vascular dysfunction were more pronounced when assessments were performed in younger women (<40 years) or closer to the index pregnancy for almost all modalities. In conclusion, pooled data from studies evaluating vascular imaging suggest that some vascular dysfunction persists following hypertensive disorders of pregnancy as compared to women with prior normal pregnancy

Key words: hypertension, preeclampsia/pregnancy, pregnancy and postpartum, endothelium, imaging, biomarkers

INTRODUCTION

Women who have had hypertensive disorders of pregnancy (HDP), including preeclampsia and gestational hypertension,[1,2] have twice the risk of subsequent cardiovascular disease (CVD) compared to women who have had normotensive pregnancies.[3,4] Furthermore, severity of HDP and fetal compromise appear most strongly associated with CVD and cardiovascular mortality.[5,6]

Vascular dysfunction, which could be pre-existing or following HDP, including endothelial dysfunction, arterial stiffness, and subclinical atherosclerosis, has been suggested as a putative mechanism for this underlying association. Preeclampsia is a complex maternal syndrome characterized by placental hypoperfusion and subsequent widespread endothelial dysfunction due to the release of inflammatory cytokines and antiangiogenic proteins.[7,8] In particular, soluble fms-like tyrosine kinase 1 (sFlt-1), an antiangiogenic protein, is elevated within the placenta and serum of preeclamptic women.[9] This protein induces endothelial dysfunction by reducing the interaction of placental growth factor (PIGF) and vascular endothelial growth factor (VEGF) with their endothelial receptors.[10] An elevated ratio of sFlt-1 to PIGF has been found to be predictive of preeclampsia.[11] Soluble endoglin (sENG) an important agent in vascular homeostasis,[12] has also been observed at elevated levels in the serum of preeclamptic women and correlates with disease severity.[13]

It remains unclear whether some degree of vascular dysfunction persists in women following pregnancies complicated by HDP beyond what is captured through traditional atherosclerotic risk factors. Several vascular imaging modalities assessing function (e.g. flow mediated dilatation, augmentation index) and structure (carotid intima-media thickness), and serum angiogenic biomarkers (e.g. sFlt-1, VEGF, PIGF) have been studied in the postpartum period. However, while some authors have reported persistent abnormalities following HDP [14-17] others have not.[18-21] A 2014 meta-analysis of observational studies on non-traditional biomarkers following HDP concluded that there was evidence of endothelial dysfunction among women with a history of HDP when compared to women with previously uncomplicated pregnancies.[22] However, emphasis in this review was on biomarkers of unclear relevance to vascular disease (e.g. serum homocysteine). Thus, the aim of the present systematic review and meta-analysis was to summarize and update evidence for vascular dysfunction at least three months following HDP,

as measured by imaging modalities and a wide variety of relevant serum biomarkers involved in angiogenesis, thrombosis, and inflammation.

METHODS

Data sources and searches

The following databases were searched for relevant studies: MEDLINE (via OvidSP 1946 to 20/05/2015; via PubMed 1946 to 20/05/2015); Embase Classic + Embase (via OvidSP 1947 to 20/05/2015); BIOSIS Previews (via OvidSP 1969 to 2015 Week 25); CINAHLPlus with Full Text (via Ebsco, 1937 to 20/May/2015); The Cochrane Central Register of Controlled Trials (via The Cochrane Library, issue 4 of 12, April 2015). The search strategies used text words and relevant indexing to answer the following question: are HDP associated with vascular dysfunction in the postpartum period? The full MEDLINE strategy (Supplemental text) was applied to all databases, with modifications to search terms as necessary. Further studies were identified in Web of Science and Scopus (16/11/2015) by examining the reference lists of included studies. Clinical Trials registries were searched to identify relevant, ongoing research. Conference proceedings from the Society of Obstetricians and Gynaecologists of Canada (http://sogc.org/) were searched from 2013-2014. The Medline strategy was rerun prior to submission (3 studies were added).

Definitions of Exposure and Outcome

The exposure of interest was any HDP including preeclampsia, eclampsia, gestational hypertension, or chronic hypertension with superimposed preeclampsia according to the current guidelines.[1,2,23] Preeclampsia was defined by a new onset of a blood > 140/90 mmHg with proteinuria > 0·3g/24h after 20 weeks of gestation, while eclampsia was the presence of seizures. Gestational hypertension was defined as a diastolic blood pressure > 90 mmHg after 20 weeks of gestation and we also included systolic blood pressure > 140 mmHg according to the most recent guidelines.[1,2,23] Superimposed preeclampsia was defined as pre-existing hypertension with new onset proteinuria > 0·3g/24h after 20 weeks of gestation. The majority of the included studies used normotensive pregnancy as the main comparison group. Four studies also included a control group of nulliparous women.[24-27] We restricted our meta-analyses to comparisons with prior normotensive pregnancy.

The outcome of interest was any form of vascular dysfunction measured by imaging modalities and serum biomarkers. We included all imaging modalities assessing various types of vascular dysfunction: endothelial dysfunction by flow mediated dilatation [FMD], forearm blood flow [FBF], laser doppler and endoPAT, arterial stiffness by pulse wave velocity [PWV] and augmentation index [AIx], and subclinical atherosclerosis by carotid intima-media thickness [cIMT]).[28-30] Only serum biomarkers directly linked to vascular function either alone or in association with preeclampsia were considered including markers of angiogenesis (sFlt-1, VEGF, PIGF, sENG), inflammation (soluble intercellular adhesion molecule-1 [sICAM-1], soluble vascular cellular adhesion molecule-1 [sVCAM-1]), and thrombosis (endothelin, and fibronectin).[9,31,32] We also included novel, yet promising, biomarkers (miRNA)[33] and growth arrest specific protein 6[34]).

Study selection

Publications were assessed for inclusion and quality in accordance with PRISMA guidelines.[35] Two independent reviewers (SGM, MO) performed the study selection using specific inclusion criteria to ensure accuracy and reproducibility. The first screening was based on titles and abstracts of identified publications. All potentially relevant studies were retrieved for full-text evaluation. Both reviewers independently evaluated the full-text articles and reasons for exclusion were recorded. Disagreement was resolved by discussion between the two reviewers and by a third reviewer (ND) as necessary. The inclusion criteria were: human studies, observational studies with a control group, HDP (exposure), and vascular dysfunction assessed at least 3 months postpartum (to allow return to pre-pregnancy physiological baseline) by imaging modalities or serum biomarkers. If duplicate studies were found within the same data source, either the most recent or the most complete publication was selected.

Data extraction

SGM and MO completed data extraction for all articles that met inclusion criteria during the full-text review. Study design and details regarding exposure, including the definition provided by each study, were recorded. The following baseline characteristics of study participants were collected: number of participants in each group, mean or median age at the time of assessment, parity, mean time since the affected pregnancy, and presence of CVD risk factors

at the time of the assessment (chronic hypertension, diabetes mellitus, cholesterol profile, prior history of symptomatic CVD, smoking status, and body mass index). The modalities used to assess vascular dysfunction (e.g. dynamic vascular imaging or blood biomarkers) with associated mean or median values for each study population were recorded. Authors of articles with insufficient study details or incomplete reported results were contacted and allotted three weeks' time for response. The characteristics of studies included in the meta-analysis are presented in Table 1 and of those included in the systematic review only in Supplemental Table S1. References of the included articles are all included in the supplemental materials.

Quality assessment

The reviewers applied the Newcastle-Ottawa Scale (NOS)[36] to assess study quality. The quality of observational studies was determined by assignment of stars to a series of questions that assess 3 categories of biases: selection, comparability, and exposure. Based on available literature, we considered age, chronic hypertension and diabetes mellitus as the most important confounders to be considered.[37-39] The NOS scale was selected as opposed to the Cochrane Risk of Bias Assessment Tool: for Non-Randomized Studies of Interventions[40] as we did not evaluate an intervention. No study was excluded on the basis of quality alone.

Statistical analysis

Results of studies were pooled using a random effects model if there were at least three studies evaluating the same technique or biomarker that reported means +/- standard deviation (SD) or standard error of mean (SEM). Results of the meta-analyses are presented as weighted mean difference (WMD) between HDP and normotensive pregnancy with the corresponding 95% confidence interval (CI). Heterogeneity was assessed using the I-squared (I^2) method. If substantial heterogeneity was present, results were only pooled if subgroup analyses reduced heterogeneity. Pre-defined subgroup analyses included stratified results based on women's age at the time of assessment (dichotomized at 40 years), the delay since the index pregnancy (dichotomized at median duration of follow-up in months for each modality), study design (cohort or case-control), type of HDP (preeclampsia, gestational hypertension, or combined), and severity of cases. For modalities examined in 10 or more studies, visual inspection of funnel plot and an Egger's test were conducted to assess for possible publications bias. A two-sided p-value

less than 0·05 was considered statistically significant for all analyses. Analyses were conducted using Stata, version 13 (StataCorp).

RESULTS

Search results and characteristics of included studies

In the initial literature search, 6109 potentially relevant studies were identified, of which 177 full-text articles were retrieved for detailed assessment (Figure 1). Sixty-five studies and seven abstracts with sufficient data were included in our final review: 59 case-control and 13 cohort studies for a total of 8702 women (3356 cases with HDP and 5346 controls). Thirty-seven studies were pooled in our analyses (Table 1). The characteristics of studies that were not pooled are presented in the Supplemental table S1.

The definitions of preeclampsia, gestational hypertension, and HDP were consistent with the guidelines of the International Society for the Study of Hypertension in Pregnancy, including the distinction between early (<34 weeks) and late (>34 weeks) preeclampsia.[23] However, one study used an unusual definition for early preeclampsia (<24 weeks),[19] as specified in Table 1.
The most frequently used modalities to assess different types of vascular dysfunction were cIMT, FMD, carotid-femoral PWV (cfPWV), AIx, sICAM-1, sVCAM-1, sFlt-1 and VEGF (Supplemental table S2), and results for these modalities were pooled if appropriate.

Imaging Modalities of Vascular Dysfunction

Measures of arterial stiffness

Arterial stiffness was assessed following any HDP using AIx in 1145 women (283 exposed and 862 controls) and more specifically following preeclampsia with cfPWV in 1087 women (242 exposed and 845 controls). Within these modalities, pooled results demonstrated persistence of arterial stiffness after the index pregnancy (AIx: WMD of 5·48% [1·58 to 9·37]), cfPWV: WMD of 0·64m/s [0·17 to 1·11]) (Figure 2). However, there was evidence of at least moderate heterogeneity measured by the I^2-method in these pooled modalities (AIx: 88·0%, cfPWV: 81·5%). We explored whether this was due to study quality, study design or type of exposure. The heterogeneity in the AIx results seemed to be explained by study design, as heterogeneity was not present in cohort studies (I^2=0·0%), but it was substantial in case-control studies (I^2=91·3%). Examining forest plots, the overall WMD for cfPWV was influenced by the

study by Tam et al., which was only available as an abstract.[41] Excluding this study, heterogeneity was diminished (I^2=0·0%), but findings of greater arterial stiffness in women post-HDP persisted. Subgroup analyses based on mean age (< or ≥ 40 years) revealed that differences in vascular dysfunction were more pronounced in younger women as measured by AIx (WMD of 6·27% [1·86 to 10·69] vs. 3·62% [-4·55 to 11·79]), and cfPWV (WMD of 0·372m/s [0·153 to 0·592] vs. 0·771m/s [0·003 to 1·538]). Visual inspection of a funnel plot and the Egger's test revealed no evidence of publication bias for AIx (Supplemental figure S1).

Measures of subclinical large vessel atherosclerosis

Persistence of subclinical large-vessel atherosclerosis in women with HDP as compared to controls was also found. Pooled analysis of cIMT measured in 802 women (341 exposed and 461 controls) revealed an overall WMD of 0·025mm [0·004 to 0·045]) (figure 2). However, there was evidence of moderate heterogeneity (50·4%) measured by the I^2-method. Results of cIMT were influenced by the study by Aykas et al.,[42] without a readily apparent explanation: the population and definition of preeclampsia were similar and the study was at low risk of bias. Nevertheless, pooled analyses excluding this study reduced heterogeneity (I^2=0·0%) without affecting the overall WMD. Subgroup analyses based on mean age (< or ≥ 40 years) were similar as with arterial stiffness modalities (WMD of 0·031mm [0·002 to 0·061] vs. 0·009mm [-0·012 to 0·029]). Visual inspection of a funnel plot and the Egger's test revealed no evidence of publication bias for cIMT (Supplemental figure S2).

The magnitude of between-group differences in various measures of vascular dysfunction (cIMT, Aix, and cfPWV) diminished over time, as might be expected based on the development of traditional atherosclerotic risk factors in the control groups as they age. (cIMT: WMD of 0·03mm [0·01 to 0·05] prior to 48 months post-delivery vs. 0·02mm [-0·02 to 0·07] after 48 months post-delivery; AIx: WMD of 9·92% [5·92 to 13·92] prior to 60 months post-delivery vs. 2·69% [-1·79 to 7·17] after 60 months post-delivery; WMD of 0·63m/s [-0·16 to 1·42] prior to 287 months post-delivery vs. 0·54m/s [0·19 to 0·88] after 287 months post-delivery). To test this hypothesis, we evaluated the absolute values in exposed and control women prior to and following the overall median follow up time (Supplemental table S3). We found that indeed, cfPWV and CIMT increased over time (eg. with age) in the control groups. This same trend was

not as apparent with Aix, but the duration of follow-up was shorter than the other marker of arterial stiffness (cfPWV).

General measures of endothelial dysfunction

There was high heterogeneity between studies assessing endothelial dysfunction through FMD, which was not reduced by our subgroup analyses. Thus, results of theses studies were not pooled.

Serum Biomarkers of Vascular Dysfunction

Markers of angiogenesis

Our included studies evaluated sFlt-1 (704 women [359 exposed and 345 controls]) and VEGF (528 women [273 cases and 255 controls]) to assess impaired angiogenesis in women with previous HDP. In pooled analyses, mean levels of sFlt-1 were modestly higher in women with previous HDP compared to women without (WMD of 6·12 pg/ml [1·91 to 10·33])(Figure 3), which represents a mean difference of 12% between women with and without prior HDP. However, pooled results revealed no significant difference between groups in mean levels of VEGF (WMD of 1·15 pg/ml [-26·12 to 28·42])(Figure 3). There was moderate heterogeneity in studies assessing sFlt-1 and VEGF (I^2=49·3% and 44·9%, respectively). We were unable to account for this heterogeneity after evaluating the impact of study quality, study design, or type of HDP. In subgroup analyses based on maternal age and delay since pregnancy, a difference was only observed for sFlt-1. Differences in mean levels of sFlt-1 post-HDP were more pronounced when measured closer to the index pregnancy (WMD of 10·44pm/ml [1·38 to 19·51] prior to 94 months post-delivery vs. 3·23pg/ml [-0·18 to 6·23] after 94 months post-delivery). We were unable to adequately assess publication bias for studies on these serum biomarkers, as there were fewer than 10-pooled studies for each marker.

Markers of inflammation

Studies measuring markers of vascular inflammation in women with and without previous preeclampsia included sICAM-1 (1010 women [541 cases and 469 controls]) and sVCAM-1 (1007 women [517 cases and 490 controls]). Pooled analyses of these biomarkers revealed similar levels in women with and without previous preeclampsia (WMD of 0·68 ng/ml [-16·12 to

17·47] and 3·94 ng/ml [-18·04 to 25·92], respectively) (Figure 3). There was moderate heterogeneity in studies assessing sICAM-1 (I^2=59·0%), but not in studies measuring sVCAM-1 (I^2=0·0%). As with the angiogenesis biomarkers, we were unable to account for the heterogeneity in sICAM-1 studies after evaluating the impact of study quality, study design, or type of HDP. In subgroup analyses based on maternal age and delay since pregnancy, no difference was observed with these biomarkers. There were not enough studies included to adequately assess publications bias.

Other modalities

Our search strategy revealed studies evaluating other markers and techniques to measure vascular dysfunction. However, pooled analyses were not conducted either because there were 3 or fewer available studies (i.e. PIGF, EndoPAT, fibronectin, sENG, miRNA, large and small artery elasticity index, growth arrest specific protein 6, ambulatory stiffness index, CD34+-VEGF-2+, CD133-VEGF-2+, and vascular compliance), or because reported results were insufficient (laser doppler, FBF, endothelin) (S2).

Quality assessment

Quality assessment results are presented in Table 1 and Supplemental Material 2, with 53·5% (38/71) of the studies at medium risk of bias (4-6 stars) and 25·3% (18/71) at high risk (0-3 stars), which is mostly due to the inclusion of abstracts. Of the 37 pooled studies, 18 (48·6%) were at medium risk of bias.

DISCUSSION

Our systematic review and meta-analysis represents the most recent and updated work summarizing the evidence for subclinical vascular dysfunction in women with prior HDP compared to women with prior normotensive pregnancies, which is a hypothesized mechanism explaining the increased risk of premature CVD in these women. We included case-control and cohort studies without language restriction, and pooled studies stratified by modality in order to reduce potential heterogeneity. We explored reasons for heterogeneity in all pooled studies. We used a validated quality assessment tool (NOS),[36] and multiple reviewers to avoid selection bias. We summarized evidence from studies evaluating various types of vascular dysfunction through

imaging modalities (arterial stiffness: cfPWV, Aix, and subclinical atherosclerosis: cIMT) as well as soluble biomarkers (angiogenesis: sFlt-1, VEGF, and inflammation: sICAM-1, sVCAM-1) in women with prior HDP. All of the pooled studies on imaging modalities demonstrated more vascular dysfunction in women with prior HDP as compared with women with prior normotensive pregnancy. In contrast, sFlt-1 was the only biomarker consistently higher in women with prior preeclampsia relative to women with recent normotensive pregnancy. Results for other biomarkers were varied and could not be pooled. To date, literature on serum biomarker involvement in angiogenesis or inflammation following HDP has been inconsistent, with reports of lower[43] or higher[21] levels in women with preeclampsia compared to normotensive pregnancy after a 3-month follow-up period. Our hypothesis is that serum biomarkers, which are responsible for the initial endothelial insult during pregnancy and are more expressed in placental tissue than in the endothelium,[9] may not be detected at elevated levels after the index pregnancy. Furthermore, commercial kits might not be sensitive enough to measure lower levels of these circulating or protein-bound biomarkers later in life. However the endothelial damage they caused during the index pregnancy is sustained and captured by other more sensitive vascular imaging modalities years following delivery.

Thus, we believe that our pooled results of vascular imaging techniques (cfPWV, cIMT, AIx) provide more robust evidence of vascular dysfunction following HDP. cIMT is a well-established marker of subclinical atherosclerosis.[44] Specifically a value of > 0·6 mm has been associated with a high risk of coronary artery disease.[45] We found a pooled WMD of 0·025mm (0·004 to 0·045), which is small but may nevertheless indicate the beginning of large-vessel atherosclerosis in women post-HDP. Furthermore, cfPWV, the gold standard measure of arterial stiffness, has been directly associated with increased risk of vascular disease and events.[29] A recent meta-analysis demonstrated that an increase of cfPWV by 1m/s corresponds to an adjusted risk increase of 14% in total vascular events after mean follow-up of 7·7 years.[46] By extension, the WMD of 0·64m/s (0·17 to 1·11) as demonstrated in our meta-analysis indicates greater arterial stiffness and a heightened risk of vascular disease in young women with prior HDP.

We found that the difference of vascular dysfunction between women with and without prior HDP was more pronounced in younger women. This may indicate that damage is present in early years following complicated pregnancy, which is generally a period where traditional CVD risk factors are not experienced.[47] This difference appeared to be attenuated with time possibly

due to the development of traditional atherosclerotic risk factors, the disparity between groups becomes less evident. Other factors that change over time may also explain this observed phenomenon. For example, older women might become more aware of CVD and choose to modify their lifestyle or start medications for CVD risk factors, both of which may have influenced the results of vascular imaging techniques.

Regardless, these finding have important potential implications for management and surveillance of young women following HDP. In an effort to prevent CVD in these women, it is currently recommended that women with HDP be screened for CVD risk factors in the postpartum period.[1,2] Our study results suggest that systematic measurement of vascular dysfunction with imaging modalities might be considered as part of global CVD screening among this population. The utilization of one of these imaging modalities could help to distinguish women with previous HDP at very high-risk (with preclinical atherosclerosis) who could benefit from a more aggressive control of CVD risk factors and specific reduction strategies even if their absolute risk of CVD is low. Furthermore, use of techniques that appear to persist regardless of age or time since delivery might prove the most appropriate choices to follow high-risk women over time. Thus, future prospective studies are required to determine whether the presence of vascular dysfunction on imaging is correlated with a greater rate of vascular events and should prompt specific vascular risk reduction therapies.

Our study contributes to the knowledge gained from the recent meta-analysis by Visser et al on CVD biomarkers following HDP.[22] In our study, we included more biomarkers directly linked to vascular function, as our hypothesis was that global endothelial dysfunction results in accelerated vascular aging and persistent dysfunction years following delivery. Additional studies on fibronectin,[14] sICAM-1,[16] sVCAM-1,[16] endothelin,[48] VEGF[49,50] and sFlt-1[49,50] are featured in our meta-analysis as our search strategy included a supplemental database (BIOSIS Previews) as well as references of the included articles. The complete search strategy of the meta-analysis by Visser et al was not available in the full text of the manuscript or the supplemental material, thus we were unable to directly compare the MeSH and non-MeSH terms used. Our meta-analysis, to our knowledge is the first to pool results of vascular dysfunction measured by validated imaging modalities, which most closely reflected the vascular endothelium function and structure.

We restricted our review to studies that assessed vascular dysfunction at least 3 months postpartum to allow time to return to pre-pregnancy physiology. We wanted to answer the

question whether events occurring during pregnancy resulted in accelerated vascular aging beyond the immediate postpartum period. The delay since pregnancy varied significantly between studies (range 3-480 months) suggesting that damage persists well beyond the immediate postpartum period in most cases, and is unlikely to completely regress spontaneously. Large-scale prospective studies would better address the trajectory of vascular dysfunction following HDP to eventually assess whether vascular dysfunction is present pre-partum in these women.

Limitations

Our review has several limitations. First, there was substantial heterogeneity in some of our results that could not be explained by the age of women at the time of assessment, study design, or time elapsed since the index pregnancy. Thus, the pooled results for those modalities should be interpreted with caution. However, heterogeneity for other results has been explained by study design (AIx) or by the results of one specific study (cIMT[42], cfPWV[41]). Second, the duration of follow-up since the index pregnancy was reported in various ways and precluded pre-planned subgroup analyses to address whether a longer or shorter delay affected the heterogeneity of some of our pooled results. However, by estimating the median duration of follow-up in months for each modality, we were able to show that, for studies assessing cIMT and AIx, the difference between both groups was more pronounced in the earlier period after the index pregnancy. This finding corroborates the results of our subgroup analyses based on age, as it shows that women have more vascular dysfunction near the index pregnancy when they usually are younger and have a less important burden of traditional CVD risk factors. The definitions of HDP, preeclampsia and gestational hypertension were mainly uniform; however, studies published after 2014 may have used slightly different definitions of preeclampsia owing to the release of new guidelines on HDP, which added systolic blood pressure >140 mmHg to diastolic blood pressure of >90mmHg, as a diagnosis criteria.[1,2] Since studies were conducted during and following pregnancy, it is unclear whether vascular dysfunction was already present pre-partum, contributing to the development of HDP. However, studies evaluating vascular function in healthy women pre-pregnancy and following those with HDP until the development of CVD many years later do not exist. Longitudinal prospective cohort studies beginning in the pre-pregnancy phase would be laborious but very informative on the trajectory between HDP and

CVD. In addition, our review did not include cardiac echocardiography since our focus was on vascular impairment A future systematic review on this topic would contribute to our understanding of overall cardiac function following HDP. Moreover, to completely evaluate the confounding effect of traditional atherosclerotic risk factors on the vascular dysfunction measurements, an individual patient analysis would be preferable, but could not be done with available data. Finally, owing to small numbers of studies for some modalities, mostly for biomarkers, we were unable to assess for publication bias. Thus, it remains possible that unpublished studies exist, which could have led us to overestimate the presence of vascular dysfunction post-HDP with imaging modalities or underestimate it with biomarkers.

Despite these limitations, we believe that our results complement previous findings and provide further evidence for vascular dysfunction following HDP. Risk stratification of women following HDP using vascular imaging modalities in addition to atherosclerotic factors might identify subgroup who would benefit from aggressive risk reduction approaches.

PERSPECTIVES

This systematic review and meta-analysis demonstrated the presence of vascular dysfunction in women with previous HDP when compared to women with previous normotensive pregnancies. Vascular imaging techniques appear to be useful tools in identifying women with vascular dysfunction in the post-partum period following hypertensive disorders of pregnancy. In contrast, serum biomarkers measured following HDP appeared not as sensitive to accurately measure underlying dynamic endothelial damage. The use of vascular imaging modalities in the postpartum period might help further define an "at risk" group to be targeted for more aggressive risk factor modification.

SOURCE OF FUNDING: Canadian Vascular Network and the Research Institute of McGill University Health Center.

DECLARATION OF INTEREST: None

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NOVELTY AND SIGNIFICANCE:

1) What is new?

This study summarizes evidence for measurable subclinical vascular dysfunction, including vascular imaging techniques and sFlt-1, months to years after pregnancies complicated by hypertensive disorders. Vascular imaging techniques appear to be useful tools to distinguish women at risk for vascular disease in the post-partum period following hypertensive disorders of pregnancy.

2) What is relevant?

There is uncertainty regarding the mechanism of increased cardiovascular disease in women who have had hypertensive disorders of pregnancy. This study provides further evidence for endothelial dysfunction following initial hypertensive injury. Further, the optimal method of screening women following hypertensive disorders of pregnancy is not known. Results of this study suggest that use of vascular imaging techniques might further define an "at risk" population in the postpartum period to be targeted for assessment of more aggressive risk factor modification.

3) Summary:

Vascular dysfunction is present in women following hypertensive disorders of pregnancy. It is currently recommended that women with a history of complicated pregnancy be screened for cardiovascular risk factors in the postpartum period. Risk stratification of women following these pregnancy complications using vascular imaging modalities in addition to measurement of usual atherosclerotic factors might identify subgroup who would benefit from aggressive risk reduction approaches.

FIGURE LEGENDS

Figure 1: Flow Diagram of The Articles Selection Process

Figure 2: Results of WMD Between Women with and without Prior HDP of Pooled Imaging Modalities

A: AIx (%); B: cfPWV (m/s); C: cIMT (mm)

***** Higher values of AIx, cIMT and cfPWV means more vascular impairment.

Figure 3: Results of WMD Between Women with and without Prior HDP of Pooled Serum Biomarkers

A: sFlt-1 (pg/ml); B: VEGF (pg/ml); C: sICAM-1 (ng/ml); D: sVCAM-1 (ng/ml)

*Higher values of sFlt-1, sICAM-1 and sVCAM-1 mean more vascular impairment, while higher values of VEGF mean less vascular impairment

Figure 1: **Flow Diagram of The Articles Selection Process**

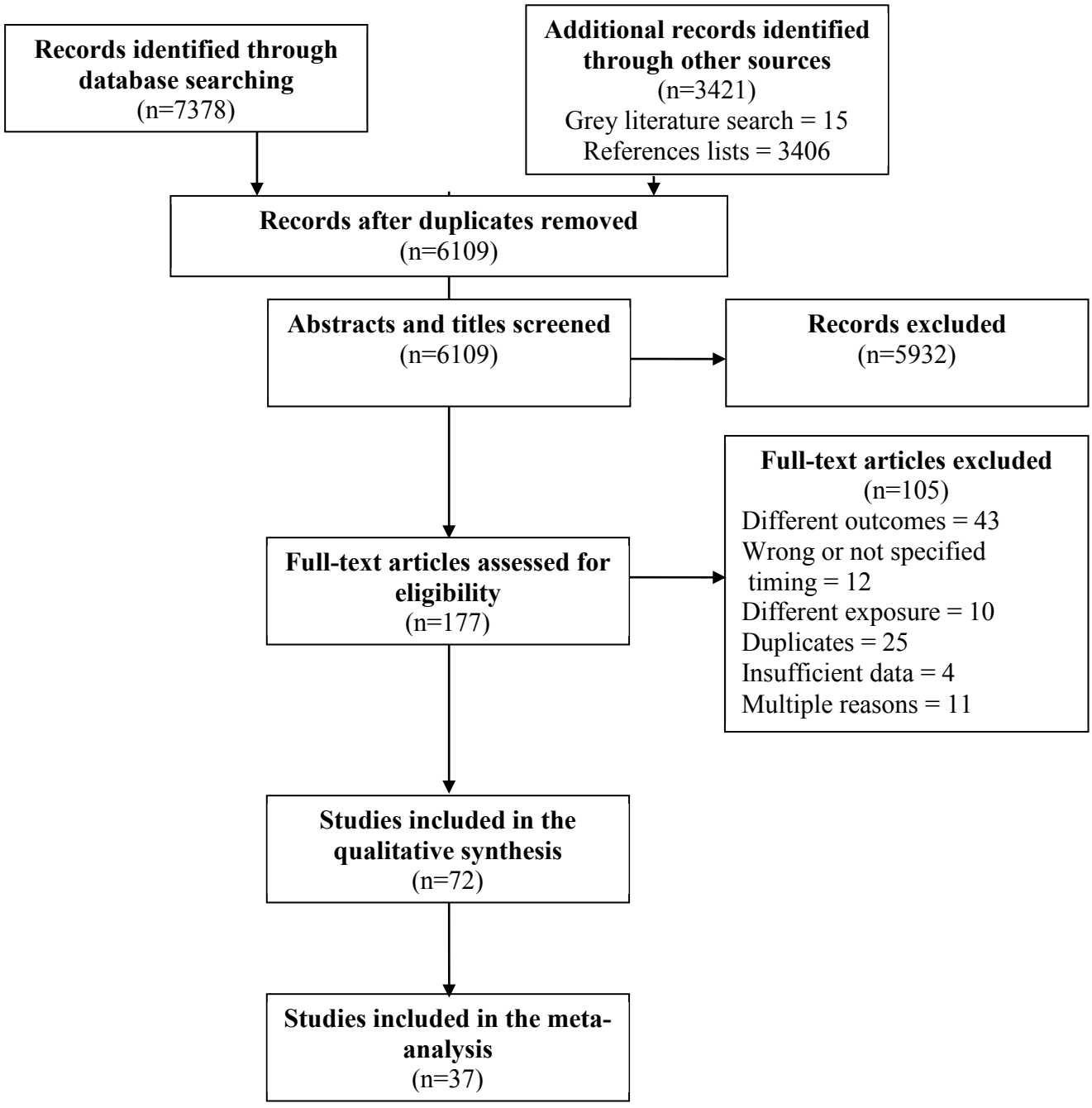
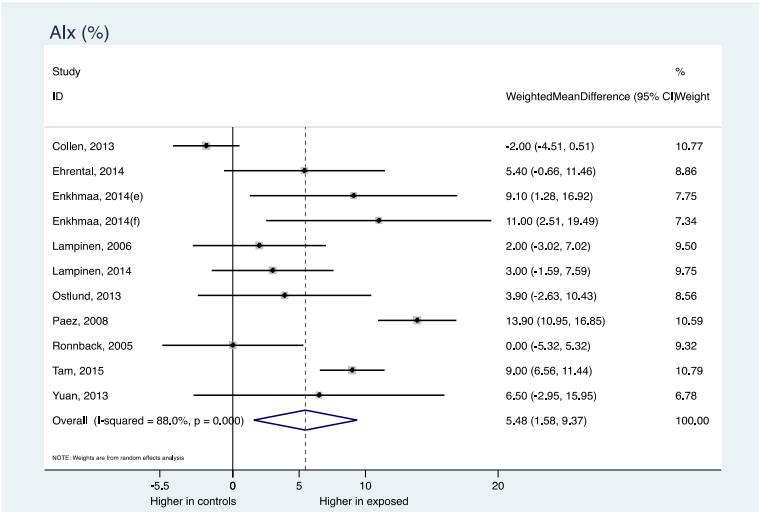
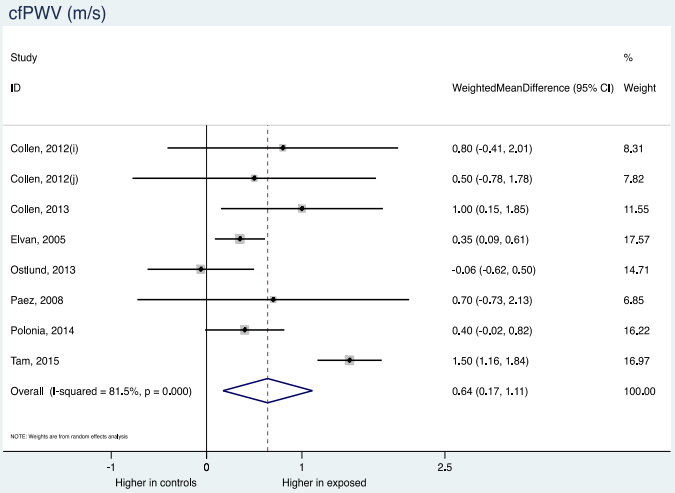
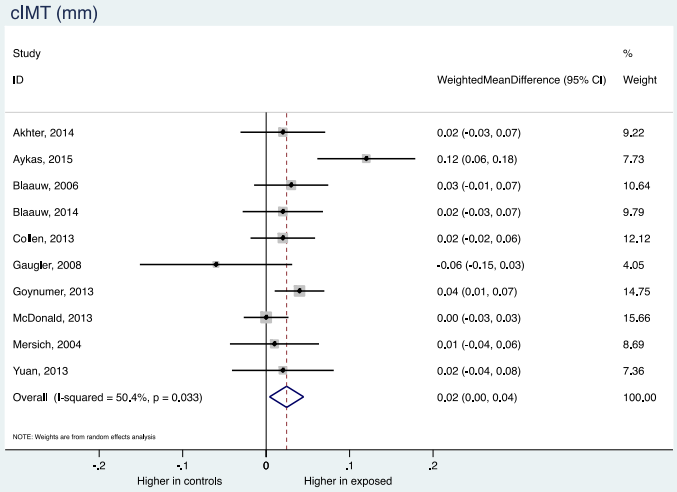


Figure 2: **Results of WMD Between Women with and without Prior HDP of Pooled Imaging Modalities**

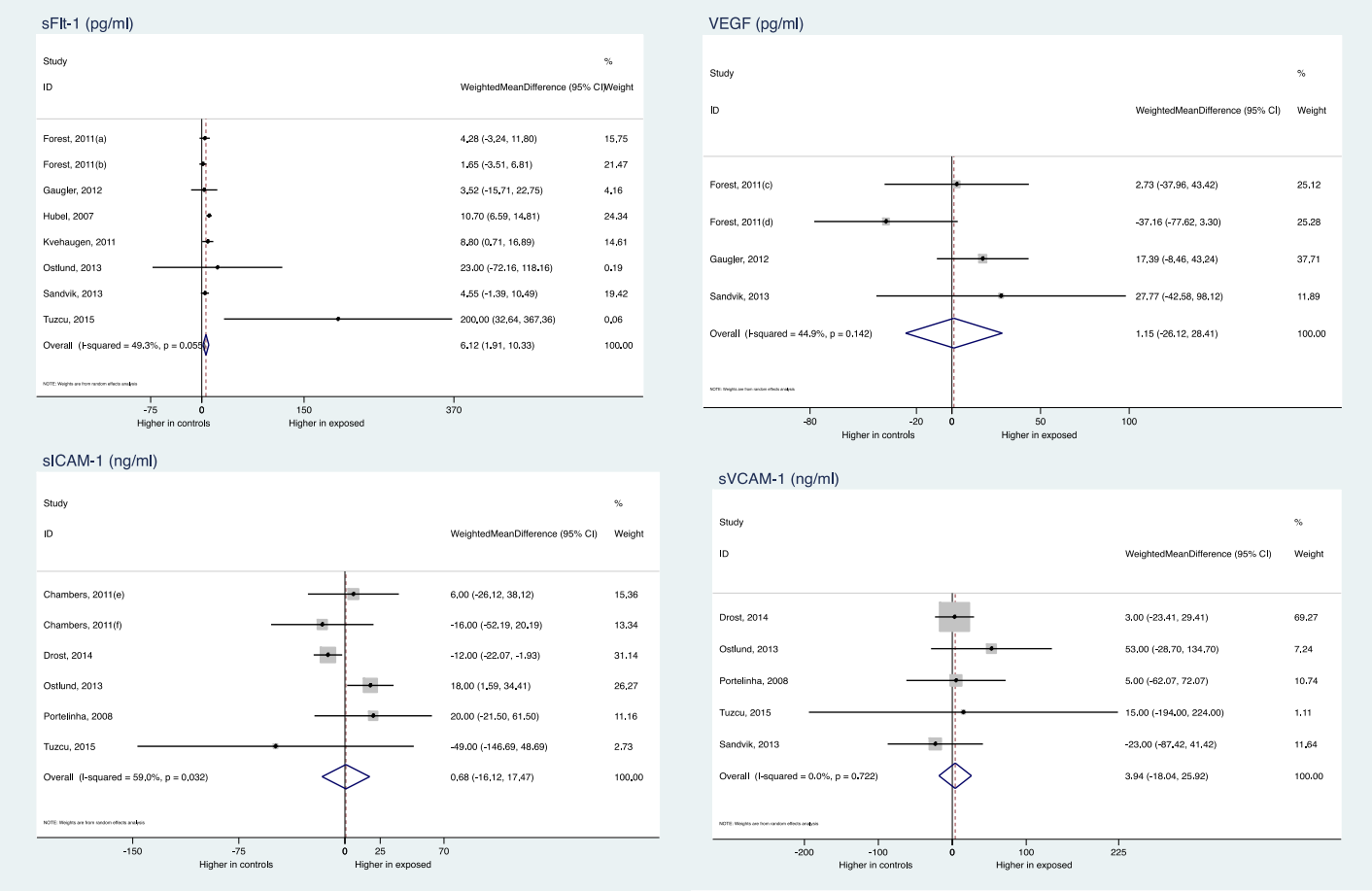






* Higher values of AIx, cIMT and cfPWV means more vascular impairment.

Figure 3: Results of WMD Between Women with and without Prior HDP of Pooled Serum Biomarkers



*Higher values of sFlt-1, sICAM-1 and sVCAM-1 mean more vascular impairment, while higher values of VEGF mean less vascular impairment

TABLE 1: Characteristics of Included Studies in the Meta-Analysis

Studies	Exposure (n)	Controls (n)	Age exposed (years)	Age controls (years)	Follow-up exposed (months)	Follow-up controls (months)	Modalities used	Quality score
Akhter, 2014[51] **(CS)**	Severe preeclampsia (42)	Normotensive (44)	44±3	44±3	132±60	132±60	cIMT	9
Aykas, 2015[42] **(CS)**	Preeclampsia (25)	Normotensive (20)	-	-	At least 60	At least 60	FMD, cIMT	8
Blaauw, 2006[25] **(CS)**	Early-onset preeclampsia (22)	Normotensive (22) / never pregnant (22)	31±4	31±4 / 30±6	6.4±2.9	7.0±2.6	cIMT	6
Blaauw, 2014[52] **(CS)**	Preeclampsia (17)	Normotensive (16)	33±5	34±4	57	52	cIMT, sICAM-1	4
Chambers,[18] **2001 (CS)**	Single (78) / recurrent (35) preeclampsia	Normotensive (48)	34±5 / 37±5	35±6	36 (median)	36 (median)	sICAM-1, FMD	7
Collen, 2012[53] **(CS)**	Previous preeclampsia and now normotensive (10) / and now with chronic hypertension (8)	Previous normotensive pregnancy and still normotensive (10)	60±5.4 / 62±4.2	63±3.1	About 480	About 480	cfPWV	5
Collen, 2013[54] **(CS)**	HDP (50)	Normotensive (55)	63±6	63±5	408 to 480	408 to 480	cfPWV, AIx, cIMT	6
Drost, 2014[43] **(CC)**	Early onset preeclampsia (339)	Normotensive (332)	38.9±4.9	39.3±4.4	109±44	128±36	sICAM-1, sVCAM-1	8
Ehrenthal, 2014[55] **(CC)**	HDP (33)	Normotensive (41)	30.4	32.0	12	12	Carotid-radial PWV, AIx	7

Elvan-Taspinar, 2005[56] **(CS)**	Early onset preeclampsia (44)	Normotensive (46)	32.4±4.8	35.1±4.1	13 (4-52) (mean range)	12 (5-46) (mean range)	cfPWV	6
Gaugler-Senden, 2008[57] **(CS)**	Severe early onset (<24 weeks) preeclampsia (20)	Normotensive (20)	38.8 (22.1-47.7)	37.7 (23.8-41.9)	66 (48-120)	70 (53-131)	cIMT	6
Gaugler-Senden, 2012[19] **(CS)**	Severe early onset (<24 weeks) preeclampsia (16)	Normotensive (18)	42.9 (38.8-45.1)	41.6 (38.8-45.7)	113 (110 – 124)	116 (112 – 131)	sFlt-1, VEGF	4
Goynumer, 2013[58] **(CS)**	Severe preeclampsia (34)	Normotensive (42)	30.94± 4.37	29.67± 4.29	19±4	19±4	cIMT, FMD	7
Hamad, 2007[20] **(CS)**	Preeclampsia (18)	Normotensive (17)	30±4	31±4	15±3	15±3	FMD, sICAM-1, sVCAM-1	8
Hamad, 2012[59] **(CS)**	Preeclampsia (35)	Normotensive (30)	-	-	5	5	FMD, PIGD, sFlt-1, VEGF, sICAM-1, sVCAM-1	8
Henriques, 2014[60] **(CC)**	Gestational hypertension (30)	Normotensive (30)	42.5±8.9	40.1±8.7	182±42	182±42	FMD	6
Hubel, 2007[15] **(CS)**	Preeclampsia (29)	Normotensive (35)	33.7±5.8	30.8±6.7	18±10	18±9	sFlt-1, VEGF	6
Kvehaugen, 2011[49] **(CS)**	Preeclampsia (26)	Normotensive (15)	37.2±4.4	40.5±4.2	77 (median)	84 (median)	sFlt-1, VEGF, PIGF, PAT,	1

Lampinen, 2006[61] (CS)	Preeclampsia (30)	Normotensive (21)	38±6	36±4	66	66	Endoglin FBF, AIx	5
Lampinen, 2014[62] (CS)	Preeclampsia (28)	Normotensive (20)	38±6	36 ±4	60 to 72	60 to 72	AIx, endothelin	6
McDonald, 2013[63] (CC)	Preeclampsia (109)	Normotensive (219)	49 (44-55)	49 (45-56)	240 (median)	240 (median)	cIMT	8
Mersich, 2004[64] (CS)	Preeclampsia (12)	Normotensive (12)	29.9±1 (SEM)	30.2±0.8 (SEM)	12±0.6	12±0.4	cIMT	4
Ostlund, 2013[21] (CS)	Severe preeclampsia (15)	Normotensive (16)	39.4±3.6	41.2±3.2	95±40	79±29	cfPWV, AIx, FMD, PIGF, sICAM-1, sVCAM-1, sFlt-1	7
Paez, 2009[26] (CS)	Preeclampsia (20)	Normotensive (20) / never pregnant (15)	24.7±4.5	22±2.7 / 25±2.6	31±4.5	31±4.6	cfPWV, AIx, FMD	2
Paradisi, 2006[65] (CS)	Gestational hypertension (15)	Normotensive (15)	34.3±1.2 (SEM)	37.6±1.5 (SEM)	20.4±1.5	20.4±1.5	FMD	7
Polonia, 2014[66] (CS)	Preeclampsia (45)	Normotensive (55)	37.8±5.1	37.9±6.4	86±48	776±34	cfPWV	5
Portelinha, 2008[67] (CS)	Preeclampsia (58)	Normotensive (49)	34 (30-39)	34 (31-39)	72 (48-96)	72 (48-96)	sVCAM-1, sICAM-1	7
Ronnback, 2005[68] (CS)	Preeclampsia (22)	Normotensive (22)	36±1 (SEM)	37±1 (SEM)	At least 60	At least 60	AIx	6
Saarelainen, 2012[69] (CS)	HDP (16)	Normotensive (24)	-	-	At least 3	At least 3	FMD	2
Sandvik, 2013[17] (CS)	Preeclampsia (89)	Normotensive (69)	37.9±4.2	39±5.3	131±12	131±12	FMD, cIMT, sVCAM-1,	6

							VEGF, sFlt-1, PIGF	
Tuzcu, 2015[70] (CS)	Preeclampsia (16)	Normotensive (24)	32.4±5.6	35.5±6.4	56.9±48	50.8±52.3	FMD, sFlt-1, sICAM-1, sVCAM-1	2
Yinon, 2010[71] (CS)	Early-onset (15) / late-onset preeclampsia (9)	Normotensive (16)	35±1 / 34±1 (SEM)	34±1 (SEM)	14±2 / 15±2 (SEM)	14±1 (SEM)	FMD	5
Yuan, 2013[72] (CS)	Late onset preeclampsia (10)	Normotensive (11)	-	-	18	18	cIMT, carotid-brachial PWV, AIx	6
			ABSTRACTS					
Enkhmaa, 2014[73] (CC)	Preeclampsia (14) / gestational hypertension (11)	Normotensive (13)	32±6 / 32±6	32±6	16.6±8 / 16±8	16.6±8	PWV, AIx	4
Forest, 2011[50] (CC)	Preeclampsia (63) / gestational hypertension (105)	Normotensive (168)	35.4 (4.9) / 34.5 (5.6)	35.4 (5.6)	94	94	sFlt-1, VEGF	3
Paez, 2010[27] (CS)	Preeclampsia (32)	Normotensive (30) / never pregnant (30)	38.2±4.7	36.7±3.9 / 36.8±4.3	120	120	FMD	0
Tam, 2015[41] (CS)	Severe preeclampsia (50)	Normotensive (643)	37.1±4.6	46.6±3.2	84±5	142±18	cfPWV, AIx	1

Results are presented as mean +/-SD or median (IQR) if not specified otherwise
AIx: augmentation index; CS: Case-control; CC: Cohort; cfPWV: carotid-femoral pulse-wave velocity; cIMT: carotid intima-media thickness; FMD: Flow mediated dilatation; FBF: Forearm blood flow; HDP: Hypertensive Disorders of Pregnancy; PAT: peripheral arterial tone; PIGF: placental growth factor; PWV: pulse wave velocity; SEM: standard error of mean; sFlt-1: soluble fms-like tyrosine kinase-1; sICAM: soluble intercellular adhesion molecule-1; sVCAM: soluble vascular cellular adhesion molecule-1;VEGF: vascular endothelial growth factor.

PREFACE TO MANUSCRIPT #2

Epidemiologic studies have demonstrated that pregnancy complications (HDP and GDM) are associated with an increased risk of CVD later in life, although the majority of these studies has used linked databases and diagnostic codes to demonstrate this association, with little granular details on the nature of the ischemic event. In fact, we know of no study that has examined the impact of these pregnancy complications at the time of an ischemic event. This unique perspective might shed light on the mechanism of vascular disease. Thus, the objective of the current study is to determine the association between prior HDP or GDM and traditional atherosclerotic risk factors as well as biomarkers of endothelial dysfunction at the time of ACS. Furthermore, we sought to compare the severity of clinical presentation and clinical outcomes at 12 months in women with premature (≤ 55 years) ACS with and without past pregnancy complications. In this study, we specifically distinguished women with prior GDM, prior gestational hypertension, and prior preeclampsia because these distinct disorders might lead to CVD through different pathophysiologic processes.

This manuscript was submitted to the American Journal of Obstetrics and Gynecology in March 2017. An abstract based on the results of this study was presented as a poster at the Canadian Women's Heart Health Summit in Ottawa in April 2016. It was also presented as an oral presentation at the Canadian Cardiovascular Congress and at the North American Society of Obstetric Medicine Annual Conference, both in Montreal in October 2016. To our knowledge, this is the first study assessing the impact of pregnancy complications at the time of a cardiovascular event.

MANUSCRIPT #2

Acute Coronary Syndrome in Women with Prior Pregnancy Complications: a Retrospective Cohort

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The authors report no conflict of interest. Table 1 should be included in the print version.
Study conducted in Canada (Quebec, British Colombia, Ontario, Alberta, Nova Scotia)

Financial support: This study was funded by the Heart and Stroke Foundation of Canada, the Canadian Institute of Health Research, and the Canadian Vascular Network. The study sponsors did not have a role in the study design, drafting of the manuscript, or data analysis.

Paper presentation information: The abstract of this work has been presented at the Canadian Cardiovascular Congress on October 22-25 2016 in Montreal, Québec Canada and at the Annual Meeting of the North American Society of Obstetrical Medicine on October 29-30 2016 in Montreal, Québec Canada.

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Word count: abstract 291; main text 3637

Women with prior pregnancy complications are younger at the time of a premature acute coronary syndrome and have a higher burden of atherosclerotic risk factors.

Short title: Pregnancy Complications and Premature ACS

Abstract

BACKGROUND: Women with cardiometabolic complications of pregnancy double the risk of cardiovascular disease. However, little data exist on the impact of these complications at the time of an acute coronary syndrome (ACS).

OBJECTIVES: To compare risk factors and clinical features among women with premature acute coronary syndrome (ACS) with or without prior pregnancy complications (gestational diabetes, hypertensive disorders of pregnancy).

STUDY DESIGN: Data were obtained from a multicentre cohort of individuals hospitalized with premature (<55 years) ACS. A total of 251 parous women were included and provided obstetric history and blood samples. They were stratified according to the presence or absence of prior pregnancy complication and followed for the development of major adverse cardiac events (MACE) at 12 months.

RESULTS: At presentation with ACS, women with prior pregnancy complication (38%) were younger than were women with prior normal pregnancy (47.4 ± 6.2 vs. 49.1 ± 5.6 years, p=0.002). They also had a higher burden of traditional atherosclerotic risk factors. Specifically, women with prior preeclampsia were more likely to have chronic hypertension (92% vs. 37% in normal group, p<0.001) and an elevated ratio of sFlt-1:PIGF (40% vs. 19%, p=0.02). They also presented more often with ST-elevation myocardial infarction (OR 2.76 [1.04, 7.29]). There was a trend for an increased risk of MACE at 12 months in women with prior pregnancy complication, mostly driven by an increased risk of recurrent ACS in women with prior preeclampsia (HR 3.46 [0.86, 13.83]).

CONCLUSION: In this cohort of women hospitalized with premature ACS, those with prior pregnancy complications were younger and had a higher burden of atherosclerotic risk factors.

Prior preeclampsia was associated with more severe ACS and a higher likelihood of recurrence. Pregnancy history should be elicited in assessment of young women with premature cardiovascular disease.

Keywords: Preeclampsia, gestational diabetes, acute coronary syndrome, major adverse cardiac events, hypertension

Introduction

Cardiometabolic pregnancy complications including the hypertensive disorders of pregnancy (HDP: preeclampsia and gestational hypertension) and gestational diabetes (GDM), affect 10% of pregnancies,[1,2] and often co-exist. In addition to causing significant maternal and fetal morbidity, these complications confer at least a 2-fold increase in the risk of premature cardiovascular disease (CVD),[3,4] as well as type II diabetes mellitus,[5,6] and chronic hypertension,[7] 10-15 years following delivery.[8,9] The magnitude of the risk due to preeclampsia is comparable to the relative risk of CVD associated with tobacco use or dyslipidemia.[10] The increased vascular risk following complicated pregnancy is thought to either be due to a high burden of traditional atherosclerotic risk factors that, in some cases, pre-dates pregnancy,[11,12] or to accelerated vascular aging following the endothelial injury initiated during pregnancy.[13] Furthermore, psychosocial factors, such as depression and anxiety, have also been reported after complicated pregnancies. These factors may contribute to an increased risk for vascular disease in women, as depression is a risk factor for mortality and cardiovascular events.[14]

Several studies have demonstrated that preeclampsia is associated with elevations in the anti-angiogenic factors soluble fms-like tyrosine kinase (sFlt-1) and soluble endoglin (sEng), and decreased levels of the pro-angiogenic placental growth factor (PIGF). These markers are associated with widespread endothelial dysfunction in pregnancy, and could potentially predict the presence or absence of preeclampsia.[15] There is uncertainty as to whether alterations in these markers persist,[16,17] reflecting sustained vascular damage and whether these alterations are a possible mechanism contributing to the development of premature overt CVD. Indeed, we recently showed in a systematic review and meta-analysis of 72 publications that vascular

dysfunction measured by imaging modalities, such as pulse wave velocity, augmentation index and carotid-intima media thickness, persists months to years after HDP.[18]

The majority of studies investigating the link between cardiometabolic pregnancy complications and future cardiovascular risk have calculated 10-year, 30-year or lifetime risks of CVD in groups of women following HDP and/or GDM,[19,20] or have used administrative databases and linked diagnostic billing codes.[21] To our knowledge and based on our literature search, no study has evaluated the impact of these pregnancy disorders at the time of an acute ischemic event. Therefore, in a multi-center cohort of young women (≤ 55 years) hospitalized with an acute coronary syndrome (ACS), we sought to compare traditional risk factors, biomarkers of endothelial dysfunction, clinical severity and major adverse cardiac events (MACE) at 12 months, in women with and without prior cardiometabolic pregnancy complications.

Methods

Study Design and Study Population

GENESIS-PRAXY (GENdEr and Sex determInantS of cardiovascular disease: from bench to beyond-PRemature Acute Coronary SYndrome) is a multicentre, prospective cohort study of young adults (≤ 55 years) hospitalized with ACS with the aim to identify sex-specific risk factors for premature CVD. Detailed methods have been previously described.[22] Briefly, participants were recruited between January 2009 and April 2013 from 24 centres across Canada, one in the US and one in Switzerland. All participating sites received ethics approval from their respective hospital ethics review boards and participants provided informed consent. Eligible participants were adults aged 18-55 years admitted to a participating hospital/coronary centre, diagnosed with ACS (see definition below), fluent in French or English, and able to provide informed consent.

Definition of Acute Coronary Syndrome

The diagnosis of ACS was determined by the treating physician based on the presence of characteristics symptoms plus at least one of the following: (1) significant electrocardiogram changes in ≥ 2 contiguous leads: transient ST-segment elevations of ≥ 1 mm, ST-segment depressions of ≥ 1 mm, new T-wave inversions of ≥ 1 mm, pseudo-normalization of previously inverted T waves, new Q waves (one third the height of the R wave or ≥ 0.04 seconds), new R > S wave in lead V1 (posterior myocardial infarction), new left bundle branch block; or (2) increase in cardiac enzymes levels: troponin I or T, creatine kinase-MB value > 2 × upper limit of the hospital's normal range or if not available, then total creatine phosphokinase value > 2 × upper limit of the hospital's normal range.[23]

Additional recorded clinical characteristics of ACS included whether there was chest pain at presentation, systolic blood pressure on arrival, peak troponin level, and the type of ACS (ST-elevation myocardial infarction [STEMI], non-STEMI [NSTEMI] or unstable angina).

Ascertainment of Prior Pregnancy Complications

Pregnancy data was ascertained at baseline via detailed self-reported questionnaire. Pregnancy history included the number of prior pregnancies, number of deliveries, and number of biological children. We also collected detailed information regarding any prior pregnancy complications. Women were asked whether, during any of their pregnancies, they received a diagnosis of gestational diabetes (GDM), high blood pressure, preeclampsia/eclampsia or whether they had protein in their urine. We then classified women as having had previous gestational hypertension if they reported high blood pressure during any pregnancy without reporting either preeclampsia or proteinuria. Women were classified as having had preeclampsia if they reported either preeclampsia or high blood pressure in addition to proteinuria. Women who were unsure about the presence or absence of a pregnancy complication or for whom

completion of these questions was incomplete were excluded from the main analyses. We estimated the time since last pregnancy using the age of the youngest biological child, to serve as a proxy for the interval between pregnancy and incident ACS.

We divided the cohort into three mutually exclusive groups (preeclampsia, gestational hypertension, and GDM) in an effort to discern distinct pathophysiological pathways of each of these pregnancy complications at the time of a premature ACS. Women with > 1 pregnancy complications were classified as having had 'any' pregnancy complication.

Biological Measurements

Venipunctures were performed for each participant within 24 hours of admission to hospital with ACS. Cholesterol levels (total, high-density lipoprotein [HDL], low-density lipoprotein [LDL]), random cortisol, Von Willebrand factor, troponin, and C-reactive protein were measured in the whole cohort. Serum and plasma were aliquoted and frozen at -80 Celcius.

Several angiogenic biomarkers (sEng, sFlt-1, and PIGF) were measured in a subgroup of women who had adequate quality never-thawed EDTA-plasma samples (n=217). PIGF and sEng were measured with the Milliplex ®MAP Human Angiogenesis / Growth Factor Magnetic Bead Panel on a Bioplex 200 system (Biorad), and sFlt-1 was measured via ELISA assay (Quantikine Human VEGFR1/sFlt-1 immunoassay; RnD systems) using the Polarstar Omega plate reader (BMG Labtech). The minimal detectable concentrations were 17 pg/ml for sEng, 0.7 pg/ml for PIGF, and 3.5 pg/ml for sFlt-1. Interassay coefficients of variation were 11% for sEng, 10% for PIGF and 12% for sFlt-1. All measurements and analyses were performed with blinding to past history of pregnancy complication as well as MACE outcome status.

Demographic and Clinical Variables

Sociodemographic data included age, household income (low if < $50 000 per annum), and education level (low if no post-secondary education). Traditional atherosclerotic risk factors

including obesity (body-mass index ≥ 30 kg/m^2), cigarette smoking status at the time of ACS, chronic hypertension, type II diabetes mellitus, dyslipidemia, familial history of CVD, and any previous vascular events (myocardial infarction, stroke, percutaneous coronary intervention [PCI], coronary artery bypass grafting [CABG] or peripheral arterial disease) were ascertained using a combination of self-report and medical chart review. Symptoms of anxiety and depression were assessed with the validated Hospital Anxiety and Depression Scale.[24] Level of stress at home was determined with a scale ranging from 1 (no stress) to 10 (most stress).[25]

Clinical Severity of ACS

The severity of the ACS was determined by type of ACS (i.e. ST-elevation myocardial infarction [STEMI] vs. other) and troponin levels. We also calculated the Global Registry of Acute Coronary Events (GRACE) score, which is a validated score used to predict in-hospital and long-term mortality or reinfarction in STEMI and NSTEMI patients.[23] The variables used to calculate the score are the age at the time of ACS, the heart rate, systolic blood pressure, creatinine and troponin level at hospital admission, the Killip class, the presence or absence of ST segment elevation and if the patient suffers from cardiac arrest.

Major Adverse Cardiovascular Outcomes at 12 Months

Participants were followed for 12 months post-ACS diagnosis to monitor for the development of MACE including cardiovascular mortality or recurrent PCI, CABG or ACS. Other outcomes included re-hospitalization for any cause, cardiac-specific re-hospitalization, and all-cause mortality.

Statistical Analyses

We compared baseline characteristics including the proportion with any traditional atherosclerotic risk factors between women with and without prior pregnancy complication (HDP and/or GDM). Dichotomous data were reported as percentages, and continuous data as means

with standard deviation or medians with interquartile ranges. Continuous data, including biomarker levels, failing Kolmogorov-Smirnov test for normality were natural log-transformed.[26] Univariate between-group comparisons were assessed using χ^2 tests and 2-sample t-tests (or Wilcoxon rank sum or Fishers exact tests as appropriate). We further conducted 3-way comparisons (preeclampsia vs. gestational hypertension vs. GDM) using ANOVA.

We compared the distribution of ACS type and troponin levels across groups and we estimated the association between prior pregnancy complications and GRACE score using univariate and multivariate linear regression. We used Cox proportional hazard models to estimate to estimate the association between pregnancy complications and occurrence of MACE, and/or each of its individual components, 12 months following ACS. All multivariate regressions were adjusted for possible confounders when possible, selected based on univariate analyses (p-value < 0.10) and a priori knowledge of predictors of GRACE and MACE.[27,28] We minimized the number of included variables in the model given our low sample size.[29] We did sensitivity analyses for the major outcomes (GRACE score and MACE) comparing all women with any pregnancy complication (n=95) to women with prior normal pregnancy. We performed sensitivity analyses whereby we considered the 59 women with insufficient data as having had normal pregnancy or complicated pregnancy.

All statistical analyses were performed using STATA version 13 (StataCorp). Statistical tests were 2-sided; differences with $p \leq 0.05$ were considered statistically significant.

Results

Study Population

Of 1,213 participants enrolled in the original cohort including 392 women,[30] we considered 310 parous women (≥1 prior pregnancies) (79%). We subsequently excluded 59

women with insufficient data on pregnancy complications. In total, we analyzed data on 251 women and 38% of them reported at least one pregnancy complication (156 women with prior normal pregnancies and 95 women with prior HDP and/or GDM). In an effort to distinguish causal pathways for each of these related but distinct pregnancy disorders, we subsequently created three mutually exclusive groups of women based on the specific type of past pregnancy complications (n=26 with preeclampsia, n=33 with gestational hypertension, n=19 with GDM, and n=17 with HDP and GDM) (Figure 1).

Burden of Traditional Atherosclerotic Risk Factors According to Pregnancy History at the Time of ACS

Compared with women with normal pregnancy, women with prior complicated pregnancy were younger at the time of ACS (47.4 ± 6.2 vs. 49.1 ± 5.6 years, p=0.002). Correspondingly, women with prior complicated pregnancy had an obstetric delivery more recently (17.4 ± 8.7 vs. 20.4 ± 7.6 years, p=0.01) (Table 1). Women with prior complicated pregnancy also had a higher burden of traditional atherosclerotic risk factors at the time of ACS diagnosis than did women with prior normal pregnancy. In fact, the odds of having at least one traditional CVD risk factor was 11-fold higher in women with prior pregnancy complications compare to women with prior normal pregnancy after adjustment for age and previous vascular events (adjusted OR 11.64 [2.40, 56.41]). For example, women with prior complicated pregnancy were more likely to be obese (56% vs. 37%, p=0.003), and more likely to have a diagnosis of either type II diabetes mellitus (38% vs. 15%, p<0.001) or chronic hypertension (80% vs. 37%, p<0.001) than were women with prior normal pregnancy. They were also more likely to have already sustained a prior vascular event (37% vs. 24% in women with prior normal pregnancy, p=0.04). Psychosocial risk factors were similar between groups, except for a higher level of stress at home reported in women with prior pregnancy complications compared to women with prior normal pregnancy

(6.0 ± 2.4 vs. 5.2 ± 2.7, p=0.01). The prevalence of depression and anxiety was not different between groups (Table 1).

In Table 1, we also present differences in key characteristics according to specific type of pregnancy complication. Women with prior preeclampsia were younger than were women with normal pregnancy, and in fact were the youngest of all groups at time of ACS (46.1 ± 6.7 vs. 49.1 ± 5.6 years, p=0.002). Nearly all women with prior preeclampsia had a diagnosis of chronic hypertension at the time of the ACS (92% vs. 37%, p=<0.001). However, women with prior preeclampsia did not appear to have a greater burden of other atherosclerotic risk factors. In contrast, women with prior gestational hypertension were more likely to be obese (72% vs. 37%, p<0.001), to have had more previous vascular events and to have type II diabetes mellitus, and chronic hypertension than women with prior normal pregnancy at the time of the ACS. Women with prior GDM were more likely to have type II diabetes mellitus (58% vs. 15%, p=<0.001) and chronic hypertension, and reported higher level of stress at home than women with prior normal pregnancy. Three-way comparisons across complications revealed differences in the proportion with obesity (p=0.03), type II diabetes mellitus (p=0.05), and chronic hypertension (p=0.05).

Biomarker Levels According to Prior Pregnancy History at the Time of ACS

All women in our cohort had similar lipid profiles and levels of cortisol, C-Reactive protein and Von Willebrand factor at the time of ACS (Table 2). Levels of angiogenic biomarkers were also similar in women with prior preeclampsia, gestational hypertension or normal pregnancy. However, women with prior preeclampsia were most likely to have an sFlt-1:PIGF ratio above the 75th percentile (38%, 27% and 19% in preeclampsia, gestational hypertension and normal, respectively, p=0.05), indicating a greater proportion with an imbalance of anti-angiogenic compared with angiogenic markers at the time of ACS.

Severity of ACS According to Pregnancy History

Women with prior preeclampsia were more likely to present with STEMI compared with women with prior normal pregnancy after adjustment for hypertension, smoking status and familial history of CVD (adjusted OR 2.76 [1.04, 7.29]), and compared with women who had other pregnancy complications (Figure 2). Furthermore, none of the women with prior preeclampsia had unstable angina as a type of ACS. Correspondingly, a greater proportion of women with prior preeclampsia had troponins in the highest tertile, although this difference was not statistically significant (Table 2). In our sensitivity analyses, there was an increase risk of STEMI in all women with prior pregnancy complications (n=95) compared to women with prior normal pregnancy after similar adjustment (adjusted OR 1.80 [1.00, 3.23], p=0.05) and this risk becomes statistically significant (p=0.044) when we added the unclassified women in the complicated pregnancy category.

Clinical severity as measured by the GRACE score at presentation was similar in all pre-defined sub-groups (71 ± 16, 71 ± 20, 72 ± 18 and 73 ± 34 in preeclampsia, gestational hypertension, GDM and normal, respectively). Similar results were obtained in our sensitivity analyses including all women with prior pregnancy complications and including women excluded for insufficient data.

Association Between Prior Complicated Pregnancy and MACE at 12 Months

At 12 months, MACE occurred in 20 women. Despite this overall low incidence in our cohort, we observed a trend for a higher rate of MACE among women with prior preeclampsia as compared to women with prior normal pregnancy (13% vs. 8%, p=0.473) (Table 3) This increase risk of MACE at 12 months was mostly driven by an increased risk of recurrent ACS (crude HR 3.46 [0.86, 13.83]), which was seen most among women with prior preeclampsia (Figure 4). Small number of events precluded the ability to adjust for hypertension or other factors. There

was no death at 12 months in this cohort of young women. Our sensitivity analyses including all women with pregnancy complications and those excluded for insufficient data did not elicit a significant relationship between pregnancy complications and the risk of MACE at 12 months.

Discussion

In this multicentre cohort of young women hospitalized with ACS, a significant proportion reported prior pregnancy complication (38% of our cohort), confirming the importance of eliciting this history in the assessment of young women with vascular disease. Our study supports previous findings that HDP and GDM are each associated with an increased risk of CVD later in life,[3,8] a risk that is similar to other traditional atherosclerotic risk factors.[10] We found that a history of prior complicated pregnancy distinguished women with a higher burden of atherosclerotic risk factors with ACS diagnosed at a younger age. Further comparisons revealed a possible difference in underlying mechanism contributing to premature vascular disease between women with prior preeclampsia, prior gestational hypertension, and prior GDM. Our study also supports the idea that the occurrence of preeclampsia strongly predicts the development of chronic hypertension 10-15 years following affected pregnancy. Our study emphasizes the role of hypertension as a key risk factor CVD in women.[31]

While mean plasma levels of circulating angiogenic and anti-angiogenic factors were not different between groups of women with and without HDP, women with preeclampsia were more likely to have a ratio of sFlt-1:PIGF above the 75th percentile, which may reflect a certain degree of impaired endothelial function at the time of ACS presentation. Zeisler and colleagues recently demonstrated that the ratio of sFlt-1:PIGF, indicating a balance between anti-angiogenesis and angiogenesis, predicts the presence or absence of preeclampsia.[15] We hypothesized that the

distribution of serum angiogenic biomarkers would reflect sustained vascular damage among women with prior preeclampsia as compared with normotensive pregnancy. There are several possible reasons for a lack of effect. First, these markers are highly correlated with blood pressure,[32] so the lack of difference may have been masked in our cohort where the majority of women with prior preeclampsia had hypertension. Moreover, our recent meta-analysis of 72 studies examining markers of subclinical vascular impairment months to years following hypertensive pregnancy revealed that imaging techniques, such as pulse wave velocity and augmentation index, may be more sensitive at detecting persistent endothelial dysfunction than commercially available angiogenic protein biomarkers.[18] Unfortunately, such vascular measures were not performed in this cohort.

A key and novel finding from our study is that women with prior preeclampsia were more likely to present with STEMI than women with prior normal pregnancy, indicating a more severe presentation of ACS. Furthermore, more women with a history of prior preeclampsia experienced recurrent ACS at 12 months. Based on these observations, it appears that women with preeclampsia are not only predisposed to ischemic heart disease, but to more severe and persistent coronary disease.

Since various forms of psychopathology including depression, anxiety and stress, are more common in women who have had preeclampsia,[33] and in individuals with CVD,[34] we explored these factors in our cohort. Our data showed that women with prior pregnancy complications reported higher level of stress at home at the time of the ACS indicating another possible component of the pathophysiological pathway in the association between pregnancy complications and CVD.

Strength and Limitations

Our study has important limitations. First, pregnancy complications were ascertained retrospectively via self-report, raising the possibility of misclassification bias. However, we believe this bias to be non-differential in nature, since all participants were questioned at the time of an ischemic event, and the link between remote pregnancy complications and coronary disease is not widely known by the lay public. Furthermore, prior studies indicate good recall of past pregnancy events.[35] Second, with the available data, the exact year of the complicated pregnancy was unknown. However, we estimate the time since delivery with the age of the youngest biological child, as a crude way to estimate the shortest possible interval between pregnancy complications and CVD. We also lacked granular data on severity or recurrence of pregnancy complications. Indeed, the severity of preeclampsia, with associated fetal growth restriction or death is most strongly associated with later ischemic heart disease.[21] The temporality of chronic medical conditions of these women was also unknown. Whether chronic hypertension or diabetes mellitus preceded pregnancy is also unknown in this study, and may lead to reverse causality. However, this is a common limitation in all epidemiological studies evaluating the link between pregnancy events and CVD outcomes,[8,9,12,21] and reflects the fact that young asymptomatic women often do not seek medical attention until routine prenatal care. Finally, our sample size was relatively small and follow-up was limited, which obviated the potential to perform important subgroup analyses. For example, we were unable to assess the association between each pregnancy complication and MACE outcomes, or investigate for the mediating effect of hypertension.

Despite these limitations, our study adds incremental knowledge about the impact that prior pregnancy complications have on clinical presentation and severity of ACS, and outcomes

at 12 months in young women. This is an analysis of prospectively collected data from multiple centres of an under-studied population (individuals < 55 years of age) with ischemic heart disease. The ability to correlate past pregnancy events with detailed information on ACS characteristics including measurement of novel biomarkers is unique and provides further evidence for the mechanism by which pregnancy complications increase vascular risk. Available evidence on this topic comes from large-scale epidemiological studies using administrative health databases that are prone to misclassification of outcomes, and where detailed information on cardiac events and details on severity at time of event are often lacking.

In conclusion, in a population of young women hospitalized with ACS, a history of prior cardiometabolic pregnancy complication was associated with younger age at presentation, and a higher burden of atherosclerotic risk factors. A history of prior preeclampsia is highly correlated with hypertension and is associated with more severe disease at presentation and a higher likelihood of recurrent ACS. Recently, cardiology guidelines have incorporated pregnancy history in assessment of women for vascular disease risk factors.[36] Future clinical trials should be aimed at targeted risk reduction in this young and vulnerable population to lessen the overall burden of CVD in women.

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Figures Legends

Figure 1: Study Flow

Figure 2: Type of ACS, Stratified by Pregnancy History

* $p<0.05$ compare to normals, adjusted for hypertension, smoking status and family history of cardiovascular disease

NSTEMI: non-STEM;I STEMI: ST-elevation myocardial infarction

Figure 3: Level of Novel Angiogenic Biomarkers, Stratified by Pregnancy History

I: PIGF (pg/ml); II: sEng (pg/ml); III: log sFlt-1 (ln pg/ml); IV: log sFlt-1:PIGF

Figure 4: MACE at 12 Months, Stratified by Pregnancy History

* $p<0.05$ compared to normals

ACS: Acute coronary syndrome; PCI: Percutaneous coronary intervention

Figure 1: Patients Flow Chart

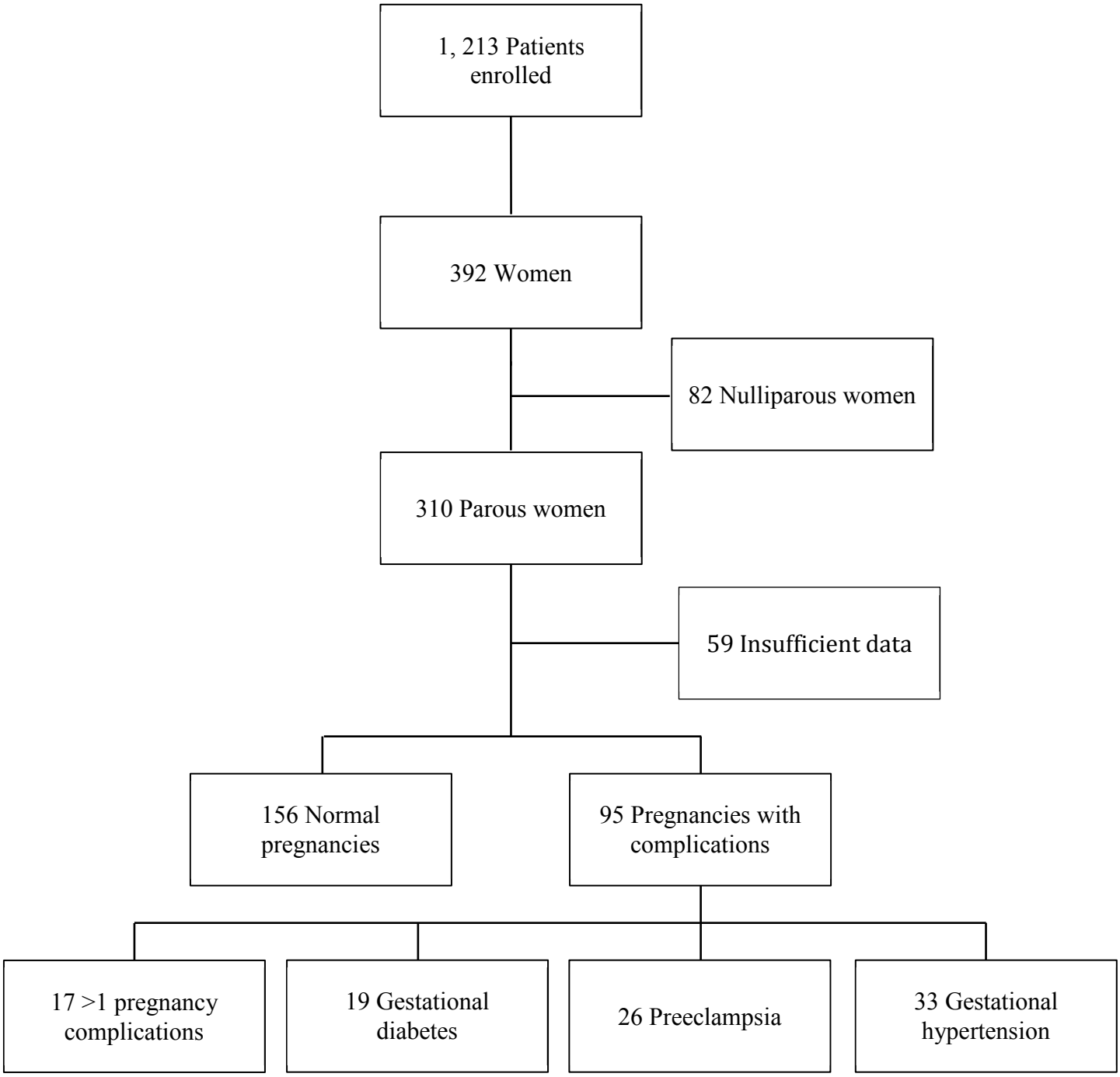

Figure 2: Type of ACS, Stratified by Pregnancy History

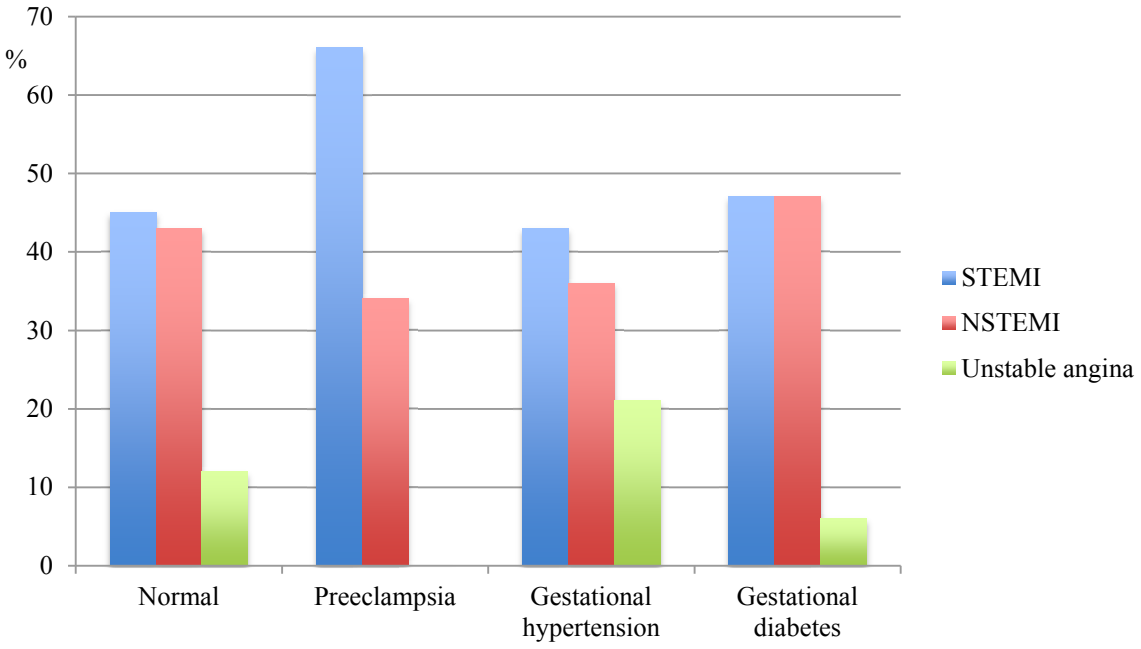

Figure 3: Level of Novel Angiogenic Biomarkers, Stratified by Pregnancy History

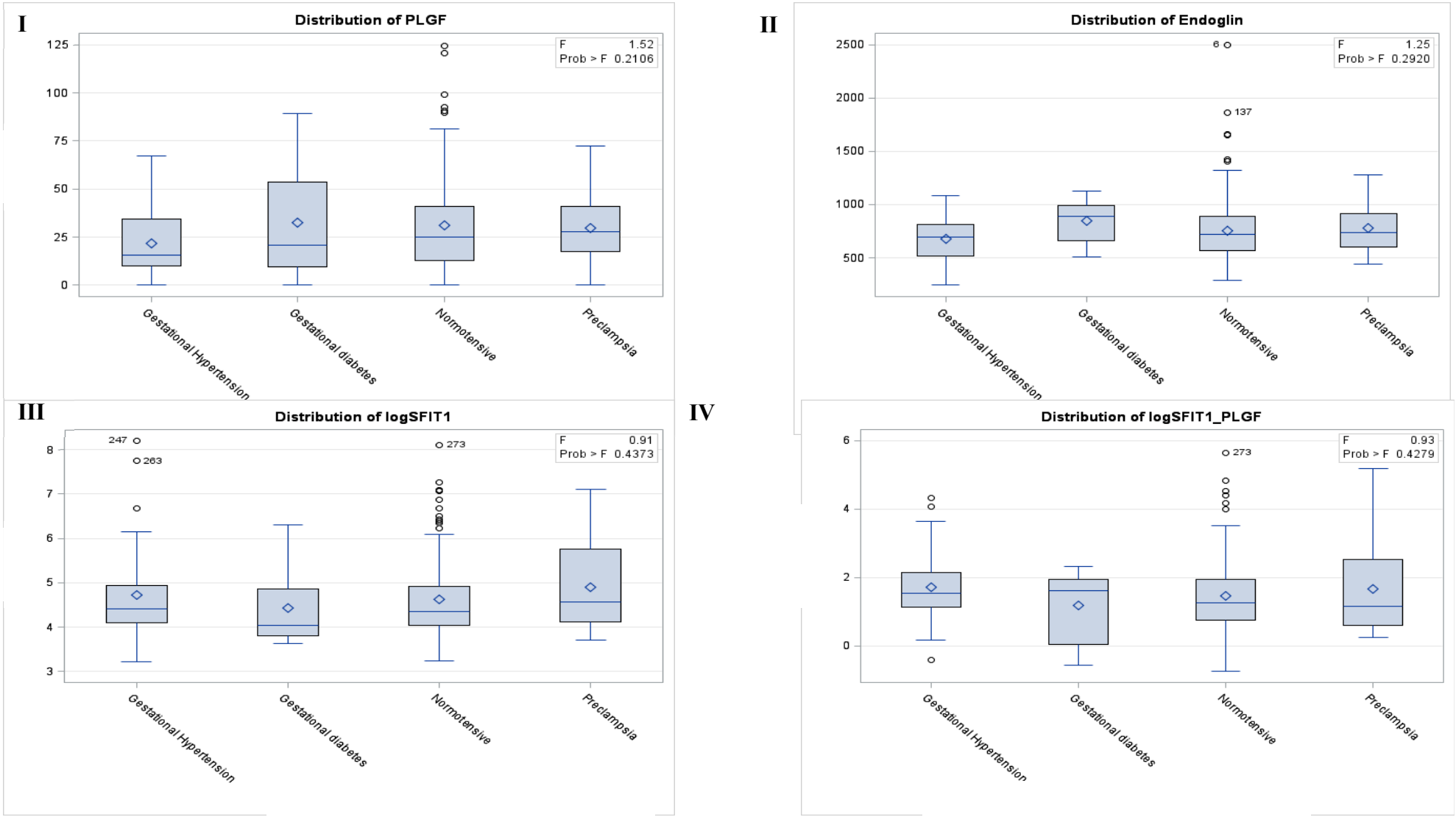

Figure 4: MACE at 12 Months, Stratified by Pregnancy History

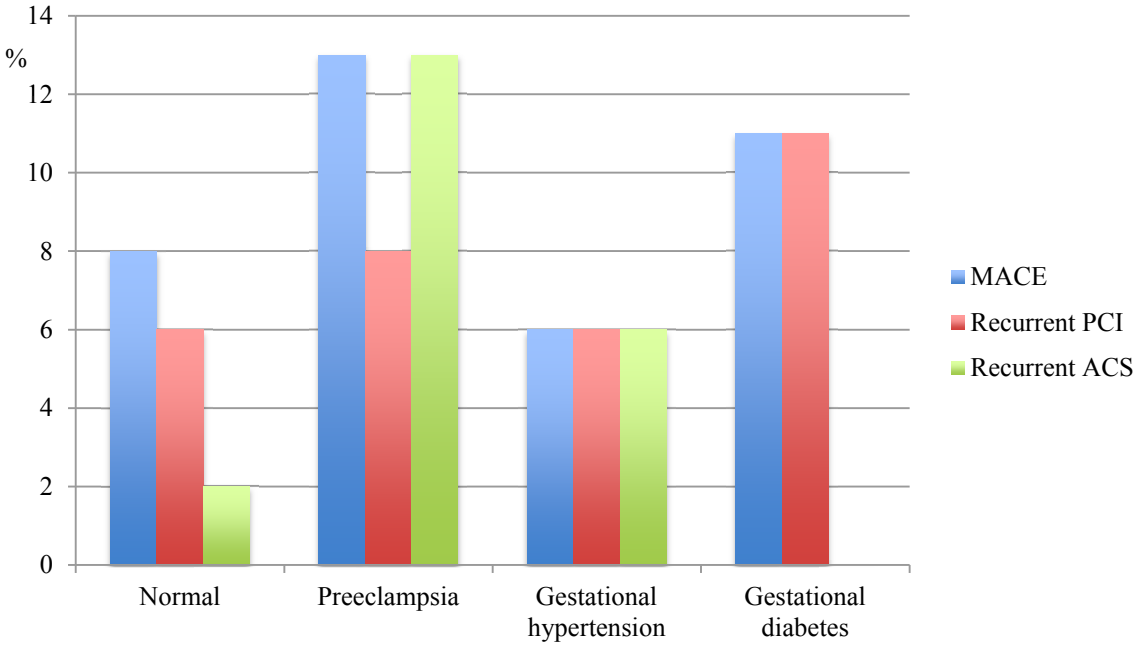

Table 1: Cardiovascular Risk Factors at the Time of the ACS Stratified According to the Presence or Absence of a Prior Pregnancy Complication

	Prior normal pregnancy (n=156)	Prior pregnancy complications* (n=95)	Prior preeclampsia (n=26)	Prior gestational hypertension (n=33)	Prior gestational diabetes (n=19)
Age (years)	49.1 ± 5.6	47.4 ± 6.2**	46.1 ± 6.7**	47.9 ± 6.3	49.5 ± 4.6
Low education[†]	72 (46)	39 (41)	5 (19)**	14 (42)	13 (68)
Number of pregnancies	2 (1)	2 (2)	3 (2)	2 (1)	2 (2)
Time since last pregnancy (years)	20.4 ± 7.6	17.4 ± 8.7**	15.4 ± 8.9**	21.1 ± 8.8	18.9 ± 5.7
Traditional Risk Factors					
Familial history of CVD	33 (21)	30 (32)**	9 (35)	13 (39)**	5 (26)
Obese[ǂ]	56 (37)	51 (56)**	11 (45)	23 (72)**	7 (37)
Current smokers	70 (45)	40 (42)	9 (35)	18 (55)	7 (37)

Previous vascular events[§]	38 (24)	35 (37)**	9 (35)	15 (45)**	3 (16)
Type II diabetes mellitus	23 (15)	36 (38)**	6 (23)	11 (33)**	11 (58)**
Chronic hypertension	57 (37)	76 (80)**	24 (92)**	26 (79)**	12 (63)**
Dyslipidemia	77 (49)	56 (59)	17 (65)	16 (48)	10 (53)
Psychosocial Risk Factors					
Low household income[‖]	58 (44)	47 (55)	12 (48)	15 (52)	10 (67)
Depression	39 (25)	26 (27)	9 (35)	7 (21)	4 (21)
Anxiety	79 (51)	53 (56)	13 (50)	17 (52)	12 (63)
Level of stress at home (scale 1-10)	5.2 ± 2.7	6.0 ± 2.4**	5.6 ± 2.8	5.9 ± 2.3	6.5 ± 2.4**

Continuous variables are presented as mean ± SD for normally distributed variables or as median (IQR) for non-normally distributed variables. Categorical variables are presented as n (%).*Pregnancy complications include preeclampsia, gestational hypertension and gestational diabetes. ** $p<0.05$, compared to the normals

ACS: Acute coronary syndrome; CVD: Cardiovascular disease. Low education[†]: no post-secondary education; Obese [ǂ]: Body Mass Index ≥ 30 kg/m^2; Previous vascular events[§]: myocardial infarction or percutaneous coronary intervention or coronary artery bypass graing or stroke or peripheral vascular disease; Low household income[‖]: < $50 000 per annum.

Table 2: Traditional and Novel Biomarkers at Time of ACS, According to Type of Prior Pregnancy Complication

	Prior normal pregnancy (n=156)	Prior pregnancy complications* (n=95)	Prior preeclampsia (n=26)	Prior gestational hypertension (n=33)	Prior gestational diabetes (n=19)
Total cholesterol (mmol/l)	4.58 ± 1.36	4.30 ± 1.36	4.21 ± 1.49	4.22 ± 1.04	4.57 ± 1.52
LDL (mmol/l)	2.75 ± 1.25	2.46 ± 1.25	2.36 ± 1.44	2.48 ± 0.93	2.68 ± 1.50
HDL (mmol/l)	1.08 ± 0.32	0.97 ± 0.37	1.09 ± 0.50	0.93 ± 0.30	0.92 ± 0.31
Cortisol (nmol/L)	284 (213)	269.0 (197.0)	252 (232)	281 (133)	274 (109)
C-Reactive protein (mg/L)	7 (15)	9.45 (17.1)	7 (13)	10 (12)	15 (25)
Von Willebrand factor (U/ml)	1.6 (0.7)	1.57 (0.67)	1.6 (0.6)	1.4 (0.4)	1.6 (0.5)
Troponin tertile					
0	14 (10)	9 (10)	3 (12)	4 (13)	2 (10)
1	63 (44)	30 (35)	7 (28)	9 (30)	8 (45)

2	34 (24)	21 (24)	7 (28)	8 (27)	3 (17)
3	33 (22)	26 (30)	8 (32)	9 (30)	5 (28)
sEng (pg/ml)	757.4 ± 310.5	728.4 ± 226.6	779.2 ± 241.6	676.5 ± 213.0	848.41 ± 189.80
sFlt-1 (ln pg/ml)	4.6 ± 1.0	4.7 ± 1.0	4.9 ± 1.0	4.7 ± 1.1	4.43 ± 0.82
PIGF (pg/ml)	31.0 ± 24.2	26.3 ± 19.2	29.6 ± 17.0	21.7 ± 16.5	32.54 ± 30.76
sFlt-1:PIGF	1.5 ± 1.1	1.6 ± 1.1	1.7 ± 1.8	1.7 ±1.1	1.18 ± 1.04
sFlt-1:PIGF >75th percentile	29 (19)	24 (29)	10 (40)**	9 (28)	1 (9%)

Continuous variables are presented as mean ± SD for normally distributed variables or as median (IQR) for non-normally distributed variables. Categorical variables are presented as n (%).
*Pregnancy complications include preeclampsia, gestational hypertension and gestational diabetes. ** $p<0.05$, compared to the normals . ACS: Acute coronary syndrome.

Table 3: Clinical Outcomes at 12 months, Stratified by Type of Pregnancy Complication

	Prior normal pregnancy (n=156)	Prior pregnancy complications* (n=95)	Prior preeclampsia (n=26)	Prior gestational hypertension (n=33)	Prior gestational diabetes (n=19)
All cause mortality	1 (0.7)	1 (1)	0	1 (3)	0
MACE	13 (8)	8 (9)	3 (13)	2 (6)	2 (11)
Cardiac mortality	1 (0.7)	0	0	0	0
Recurrent PCI	9 (6)	6 (7)	2 (8)	2 (6)	2 (11)
Recurrent CABG	2 (1)	0	0	0	0
Recurrent ACS	3 (2)	6 (7)	3 (13)**	2 (6)	0
All cause rehospitalization	23 (15)	17 (19)	4 (17)	9 (28)	2 (11)
Cardiac rehospitalization	12 (8)	11 (12)	4 (17)	5 (16)	0

Continuous variables are presented as mean ± SD. Categorical variables are presented as n (%).
*Pregnancy complications include preeclampsia, gestational hypertension and gestational diabetes.
** $p<0.05$, compared to the normal
ACS: Acute coronary syndrome; CABG: Coronary artery bypass grafting; MACE: Major adverse cardiac events; PCI: Percutaneous coronary intervention

THESIS DISCUSSION

In this thesis, we studied the association between pregnancy complications and CVD. Cardiometabolic pregnancy complications, HDP and GDM, have been linked to CVD later in life either due to a high burden of traditional atherosclerotic risk factors and/or initiation and persistence of vascular dysfunction.[24] In our systematic review and meta-analysis, we collected results of 72 articles and summarized the evidence of sustained vascular dysfunction, at least three months after HDP, measured by imaging modalities and serum biomarkers.[94] We found evidence of persistent vascular dysfunction using non-invasive vascular imaging techniques measuring arterial stiffness and subclinical atherosclerosis, and also with circulating biomarkers (notably, the anti-angiogenic biomarker sFlt-1). We hypothesized that these circulating serum biomarkers reflect widespread acute endothelial dysfunction during affected pregnancy, but may not accurately reflect chronic subclinical vascular dysfunction that persists afterwards. However, available vascular imaging modalities are more sensitive in detecting these subtle yet important changes. Interestingly, our sensitivity analysis demonstrated that the between-group differences in measures of vascular impairment were more pronounced in younger women (< 40 years), when traditional atherosclerotic risk factors are usually less prevalent.[95] As the prevalence of chronic hypertension and metabolic syndrome increases with age, we hypothesize that the differences in subclinical vascular disease will be less important in women with and without past HDP. Indeed, our findings have greatest relevance to young women following HDP. Available clinical practice guidelines recommend close follow-up and screening for traditional CVD risk factors after HDP.[33,44] We believe based on the results of our systematic review that the use of these non-invasive vascular imaging modalities in clinical practice might identify young women with persistent impairment who warrant closer follow-up and consideration for aggressive risk factor management.

In order to evaluate the consequences of pregnancy complications at the time of an ACS, we used the GENESIS-PRAXY prospective cohort by including only women who reported at least one prior pregnancy. We evaluated the differences between women with prior normal pregnancy (n=156) and those with prior pregnancy complications (n=95) (HDP and/or GDM) at the time of a premature ACS (≤ 55 years). We assessed variations in risk factor profiles, biomarkers of vascular dysfunction, severity of clinical presentation, and outcomes at 12 months

between those two groups. Our study showed that even if women with prior pregnancy complications were younger at the time of their ACS, their prevalence of traditional CVD risk factors was higher. Our results are consistent with the previous literature, which reported higher prevalence of traditional CVD risk factors years after pregnancy, in women with prior cardiometabolic complications.[57,60] Almost all women with preeclampsia had chronic hypertension at the time of the ACS, which was their main traditional risk factor. This finding confirms the strong association between preeclampsia and the development of chronic hypertension later in life. Our study also demonstrated that women with prior preeclampsia were more likely to present with STEMI than women with prior normal pregnancy, indicating more severe disease. According to this finding, women with prior preeclampsia were also more likely to have a recurrent ACS at 12 months.

At the time of their ACS, women with previous HDP had similar levels of investigational biomarkers related to preeclampsia. However, women with prior preeclampsia were more likely to have a ratio of sFlt-1/PIGF higher that the 75th percentile. These findings corroborate the results of our meta-analysis, as biomarkers seem to cause sustained vascular dysfunction during pregnancy affected by preeclampsia, damage that can be measured with imaging modalities years later, while these biomarkers are less implicated in potentiating downstream vascular disease.

Contributions to the literature

We believe that this thesis complements current knowledge regarding the association between pregnancy complications and CVD. We provided evidence of persistent subclinical vascular dysfunction months to years following HDP, mostly when measured with imaging modalities. Moreover, we showed that in a population of women with ACS, those who have had prior cardiometabolic pregnancy complications were younger at the time of an acute ischemic event, had a higher burden of traditional atherosclerotic risk factors and presented with more severe disease. As part of a comprehensive strategy to reduce the burden of CVD in women, the American and Canadian Obstetric Guidelines[33,34] suggest screening and surveillance of women after HDP or GDM.[33,34,44] Moreover, the most recent Canadian Lipid Guidelines now identify women with prior cardiometabolic pregnancy complications as a high-risk subgroup.[96]

Our first study suggests that systematic measurement of vascular dysfunction with imaging modalities might be considered as part of global CVD screening in the postpartum period. The utilization of one of these imaging modalities could help to distinguish women with previous HDP at very high-risk (with preclinical atherosclerosis) who could benefit from a more aggressive control of CVD risk factors and specific reduction strategies despite a low calculated absolute risk for CVD. Our second study corroborates findings from the first study, and suggests that hypertension should be emphasized as an important risk factor for premature CVD in women who have had HDP, and supports further research on individualized care for women at the time of an acute ischemic event.

Thesis Limitations

Biases in Meta-Analyses of Observational Studies

There was substantial heterogeneity that could not be explained by our predetermined sub-groups analyses and some of our pooled results should be interpreted with caution. Publication bias could also not be explored with some of our modalities and negative studies might have been missing and our results overestimated. The exclusion criteria were different between studies, which precludes the complete evaluation of the confounding effect of traditional atherosclerotic risk factors on the vascular dysfunction measurements.

Biases in Observation Studies with Retrospective Ascertainment of Exposure

1. Information bias

As our exposure to pregnancy complications was assessed retrospectively through self-reported questionnaire, misclassification of pregnancy events is possible. However, we think that any information bias would have been non-differential, as the link between the exposure and the outcome is not well known in the general population, and the outcome occurred in the entire cohort. Thus, recall bias is unlikely, but a general over-reporting of pregnancy complications is possible.

2. Selection bias

Cohort studies usually have less selection bias than case-control studies. Indeed, we do not think that important selection bias have influenced our results. As the initial study was not designed to evaluate the impact of pregnancy complications on CVD, the cohort entry could not have been influenced by our exposure of interest. We also had very few losses to follow-up at 12

months and, as the exposure happened many years before, it would be unexpected that the exposure influenced the proportion of losses in each group.

3. Confounding bias

Non-randomized trials are prone to bias due to residual confounding. Both known and unknown confounders might account for observed associations. However, due to our low number of events, it was not possible to adjust for all confounders.

4. Missing data

Missing data can be problematic in observational as well as randomized studies especially if data are missing not at random. Fortunately, our main analyses were not affected by substantial missing data, so this was unlikely a major contributor of bias in our study.

5. Limitations due to Type 1 and Type 2 error

The GENESIS-PRAXY study was not designed to specifically answer our question and was thus under-powered to find significant between-group differences in all outcomes, in particular MACE. Thus, there remains a possibility of a type 2 error that is not modifiable with the current design. However, the trends that we observed are compatible with what is known from prior larger scale studies, and is biologically plausible. Thus, we feel confident that the cautious conclusions rendered were appropriate.

CONCLUSION

Women with prior pregnancy complications (HDP and/or GDM) are at higher risk of premature CVD. Sustained vascular dysfunction after HDP is implicated in this phenomenon, as is the high prevalence of traditional CVD risk factors in these women. Hypertension seems to be the most widespread risk factor in these women at the time of the ACS and especially in those with prior preeclampsia. Women with prior preeclampsia also seem to suffer from more severe coronary disease and appear to be at higher risk of recurrent ischemic event. These women might benefit from in depth assessment of CVD risk after pregnancy by not only screening for traditional atherosclerotic risk factors, but also by using non-invasive vascular imaging techniques. Future studies that prospectively monitor markers of vascular impairment in women following preeclampsia are needed in order to identify subgroups at highest risk. Early intervention trials are urgently needed in this population to reduce risk of premature vascular disease in women.

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MARKERS OF VASCULAR DYSFUNCTION AFTER HYPERTENSIVE DISORDERS OF PREGNANCY: A SYSTEMATIC REVIEW AND META-ANALYSIS SUPPLEMENTAL MATERIAL

Short title : Pregnancy Complications And Vascular Dysfunction

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Supplemental text: Medline Search Strategy

1 exp Hypertension, Pregnancy-Induced/
2 Hypertension/
3 limit 2 to yr="1970 - 2004"
4 pregnancy/
5 3 and 4
6 Pregnancy Complications, Cardiovascular/
7 limit 6 to yr="1970 - 2004"
8 ((pregnant or pregnancy or pregnancies or maternal or gestation* or proteinuria or gestosis) adj3 (hypertens* or hyper-tens* or toxemia* or toxaemia*)).tw,kf.
9 (eclamp* or preclamp* or preeclamp*).tw,kf.
10 (EPH adj3 (complex* or gestosis or toxemia* or toxaemia*)).tw,kf.
11 (PIH or PPEP).tw,kf.
12 1 or 5 or 7 or 8 or 9 or 10 or 11
13 Endothelium/
14 Epithelium/
15 limit 14 to yr="1966 - 1972"
16 exp Endothelium, Vascular/
17 capillaries/
18 limit 17 to yr="1966 - 1987"
19 Blood Vessels/
20 limit 19 to yr="1966 - 1987"
21 exp Arteries/
22 Cardiovascular Diseases/
23 Vascular Diseases/
24 13 or 15 or 16 or 18 or 20 or 21 or 22 or 23
25 (ph or us or pp or pa).fs.
26 blood flow velocity/
27 Carotid Intima-Media Thickness/
28 Laser-Doppler Flowmetry/
29 Pulse Wave Analysis/
30 exp echocardiography/
31 exp Ultrasonography, Doppler/
32 vascular stiffness/
33 elasticity/
34 limit 33 to yr="1990 - 2011"
35 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 34
36 24 and 35
37 exp biological markers/
38 ((arter* or aort* or vascular or endotheli* or endo-theli* or flow or intra-media or media*) adj3 (stiffness or distensibility or dis-tensibility or elast* or function* or dysfunction* or dilat* or resist* or thickness* or thicken* or complian*)).tw,kf.
39 (echocardiograph* or echo-cardiograph*).tw,kf.
40 (pulse wave adj2 (analys* or velocit*)).tw,kf.
41 augment* inde*.tw,kf.
42 (FMD or IMT).tw,kf.

43 ((bio or biological or biochemical or circulat* or vascular or cardiovasc* or cardio-vasc* or disease*) adj3 marker*).tw,kf.
44 biomarker*.tw,kf.
45 or/36-44
46 12 and 45
47 Epidemiologic Studies/
48 exp case-control studies/
49 Control Groups/
50 exp cohort studies/
51 cross-sectional studies/
52 Postpartum Period/
53 ep.fs.
54 (case-control* or (control* adj1 group*) or longitudinal or long-term* or retrospective* or cohort* or prospective* or cross-sectional*).tw,kf.
55 (postpartum or post-partum or puerper*).tw,kf.
56 ((after or follow* or post) adj2 (deliver* or pregnan*)).tw,kf.
57 or/47-56
58 46 and 57
59 Animals/ not (Animals/ and Humans/)
60 (animals or animal or mice or mus or mouse or murine or woodmouse or rats or rat or murinae or muridae or cottonrat or cottonrats or hamster or hamsters or cricetinae or rodentia or rodent or rodents or pigs or pig or porcine or swine or swines or piglets or piglet or boar or boars or "sus scrofa" or ferrets or ferret or polecat or polecats or "mustela putorius" or "guinea pigs" or "guinea pig" or cavia or callithrix or marmoset or marmosets or cebuella or hapale or octodon or chinchilla or chinchillas or gerbillinae or gerbil or gerbils or jird or jirds or merione or meriones or rabbits or rabbit or hares or hare or diptera or flies or fly or dipteral or drosphila or drosophilidae or cats or cat or carus or felis or nematoda or nematode or nematoda or nematode or nematodes or sipunculida or dogs or dog or canine or canines or canis or sheep or sheeps or mouflon or mouflons or ovis or goats or goat or capra or capras or rupicapra or chamois or haplorhini or monkey or monkeys or macaque or macaques or primate or primates or anthropoidea or anthropoids or saguinus or tamarin or tamarins or leontopithecus or hominidae or ape or apes or paniscus or "pan paniscus" or bonobo or bonobos or troglodytes or "pan troglodytes" or gibbon or gibbons or siamang or siamangs or nomascus or symphalangus or chimpanzee or chimpanzees or prosimians or "bush baby" or prosimian or "bush babies" or galagos or galago or pongidae or gorilla or gorillas or pongo or pygmaeus or "pongo pygmaeus" or orangutans or pygmaeus or lemur or lemurs or lemuridae or horse or horses or pongo or equus or cow or calf or bull or chicken or chickens or gallus or quail or bird or birds or quails or poultry or poultries or fowl or fowls or reptile or reptilia or reptiles or snakes or snake or lizard or lizards or alligator or alligators or crocodile or crocodiles or turtle or turtles or amphibian or amphibians or amphibia or frog or frogs or bombina or salientia or toad or toads or "epidalea calamita" or salamander or salamanders or eel or eels or fish or fishes or pisces or catfish or catfishes or siluriformes or arius or heteropneustes or sheatfish or perch or perches or percidae or perca or trout or trouts or char or chars or salvelinus or "fathead minnow" or minnow or cyprinidae or carps or carp or zebrafish or zebrafishes or goldfish or goldfishes or guppy or guppies or chub or chubs or tinca or barbels or barbus or pimephales or promelas or "poecilia reticulata" or mullet

or mullets or seahorse or seahorses or "mugil curema" or "atlantic cod" or shark or sharks or catshark or anguilla or salmonid or salmonids or whitefish or whitefishes or salmon or salmons or sole or solea or "sea lamprey" or lamprey or lampreys or pumpkinseed or sunfish or sunfishes or tilapia or tilapias or turbot or turbots or flatfish or flatfishes or sciuridae or squirrel or squirrels or chipmunk or chipmunks or suslik or susliks or vole or voles or lemming or lemmings or muskrat or muskrats or lemmus or otter or otters or marten or martens or martes or weasel or badger or badgers or ermine or mink or minks or sable or sables or gulo or gulos or wolverine or wolverines or minks or mustela or llama or llamas or alpaca or alpacas or camelid or camelids or guanaco or guanacos or chiroptera or chiropteras or bat or bats or fox or foxes or iguana or iguanas or "xenopus laevis" or parakeet or parakeets or parrot or parrots or donkey or donkeys or mule or mules or zebra or zebras or shrew or shrews or bison or bisons or buffalo or buffaloes or deer or deers or bear or bears or panda or pandas or "wild hog" or "wild boar" or fitchew or fitch or beaver or beavers or jerboa or jerboas or capybara or capybaras).ti.

61 58 not (59 or 60)
62 limit 61 to (case reports or comment or editorial or letter)
63 61 not 62
64 limit 63 to "review articles"
65 limit 63 to systematic reviews
66 63 not (64 or 65)
67 remove duplicates from 66
68 limit 67 to yr="1990 -Current"

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Table S1: Studies Characteristics of those Included in the Systematic Review

Studies	Exposure (n)	Controls (n)	Age exposed (years)	Age controls (years)	Follow-up exposed (months)	Follow-up controls (months)	Modalities used	Quality score
Agatisa, 2004[1] (CS)	Preeclampsia (16)	Normotensive pregnancy (14) / Never pregnant (20)	29±1 (SEM)	26±2 / 26±1 (SEM)	9.9±0.5 (SEM)	9.5±0.5 (SEM)	FBF	6
Akhter, 2013[2] (CS)	Preeclampsia (48)	Normotensive pregnancy (58)	30 (26-34)	30 (28-33)	12	12	cIMT	7
Anders-gaard, 2012[3] (CC)	Preeclampsia (250) / gestational hypertension (138)	Normotensive pregnancy (1778)	-	-	At least 228	At least 228	cIMT	4
Barden, 1994[4] (CS)	Preeclampsia (20)	Normotensive pregnancy (28)	27.4±1.5 (SEM)	26.8±1.2 (SEM)	6	6	Endothelin	6
Barden, 1999[5] (CS)	Preeclampsia (62)	Normotensive pregnancy (84)	27.5±0.8	27.6±0.6	6	6	Endothelin	6
Barry, 2015[6] (CS)	Severe (32) / Mild Preeclampsia (17)	Normotensive pregnancy (22)	32.5±5.9 / 35.0±7.5	34.6±4.3	17.5 (13-39) / 15 (11-35)	16.5 (13-25)	FMD	7
Berends, 2008[7] (CS)	Preeclampsia (48)	Normotensive pregnancy (100)	36.2±5.8	39.2±5.6	84±67	157±68	cIMT	5
Blaauw, 2005[8] (CS)	Severe preeclampsia	Normotensive pregnancy	29.9±4.2	32.3±2.7	7.0±2.8	6.0±2.2	Laser doppler	6

	(25)	(23)						
Bremme, 1996[9] (CS)	Severe (28) / Mild preeclampsia (14)	Normotensive pregnancy (26)	29 (21-38)/ 28 (23-34)	Range of 20-40	9 (6-15)	-	Fibronectin	1
Carty, 2012[10] (CC)	Preeclampsia (27)	Normotensive pregnancy (68)	29 (22-36)	35 (20-44)	6 to 9	6 to 9	PAT	6
Ciftci, 2014[11] (CS)	Mild preeclampsia (46)	Gestational hypertension (38)	33.28± 7.34	34.00± 9.48	63.54± 2.19	63.57± 2.77	cIMT	8
Deng, 1994[12] (CS)	Severe (21) / Mild preeclampsia (10)	Normotensive pregnancy (11)	-	-	5 to 15	5 to 15	Fibronectin	0
Estensen, 2013[13] (CC)	Preeclampsia (75)	Normotensive pregnancy (63)	32±6	32±5	6	6	Vascular compliance	5
Estensen, 2015[14] (CS)	Preeclampsia (34)	Normotensive pregnancy (61)	-	-	6	6	sVCAM-1	7
Evans, 2011[15] (CC)	Preeclampsia (18)	Normotensive pregnancy (50)	28.1±1.3	29.9±0.6	16.5±1.1	16.5±0.6	PWV, FBF, Fibronectin	6
Karkkainen, 2013[16] (CC)	HDP (15)	Normotensive pregnancy (27)	-	-	At least 3	At least 3	AASI	5
Laivuori, 1998[17] (CS)	Severe preeclampsia or eclampsia (21)	Normotensive pregnancy (20)	41.8±0.9	41.8±0.9	202±1	204±1	Endothelin	3
Lampinen, 2006[18] (CS)	Preeclampsia with	Normotensive pregnancy	37.7±7 / 38±5	36±4	60 to 72	60 to 72	FMD	6

	proteinuria <5g/d (8) / >5g/d (22)	(22)						
Lazzarin, 2012[19] (CS)	HDP (25)	Normotensive pregnancy (25)	33±5.6	32.9±4.5	3	3	sICAM-1, sVCAM-1	6
Lommerse, 2007[20] (CS)	Preeclampsia (32)	Normotensive pregnancy (10)	31 (27-34)	33 (31-36)	9 (7-19)	20 (10-40)	FBF	2
Mangos, 2012[21] (CC)	Preeclampsia (39) / Gestational Hypertension (27)	Normotensive pregnancy (35)	37±6 / 36±6	38±6	46 (30-60)/ 35 (26-72)	52 (34-84)	FBF	6
Murphy, 2013[22] (CS)	Preeclampsia (17)	Normotensive pregnancy (13)	31.5±5.4	31.8±3.7	6	6	CD34+ VEGFR-2+, CD133+ VEGFR-2+	6
Murphy, 2015[23] (CS)	Severe (6) / Mild preeclampsia (7)	Normotensive pregnancy (17)	-	-	12	12	miRNA	3
Orabona, 2015[24] (CS)	Early-onset (30) / Late-onset preeclampsia (30)	Normotensive pregnancy (30)	38±4 / 36±6	37±4	6 to 48	6 to 48	PWV	5
Ramsay, 2003[25] (CS)	Preeclampsia (10)	Normotensive pregnancy (10)	46 (40-49)	45 (43-48)	264 (192-276)	246 (216-288)	Laser doppler	1
Sattar,	Preeclampsia	Normotensive	43 (40-	44 (43-47)	At least 226	At lest 226	sICAM-1,	5

2003[26] **(CS)**	(40)	pregnancy (40)	47)				sVCAM-1	
Souwer, 2011[27] **(CS)**	Early onset preeclampsia (14)	Normotensive pregnancy (16)	33±5	34±4	55±7	52±2	Large and small artery elasticity index	3
Spaander-man, 2000[28] **(CS)**	Preeclampsia and thrombophilia (18) / and chronic hypertension (11) / and no other disease (13)	Normotensive pregnancy (10)	29±4 / 33±4 / 29±3	31±2	12 (6-46) / 11 (6-29) / 20 (6-46)	18 (6-48)	Vascular compliance	3
Spaan, 2010[29] **(CS)**	Preeclampsia (22)	Normotensive pregnancy (29)	49±3.9	49.8±3.9	276 (240-336)	276 (240-336)	Laser doppler	6
Stepan, 2013[30] **(CS)**	Preeclampsia (44)	Normotensive pregnancy (45)	31 (27-35)	30 (37-36)	312 (300-336)	324 (276-372)	Growth arrest specific protein 6	5
Tyldum, 2012[31] **(CS)**	Preeclampsia (19)	Normotensive pregnancy (19)	29±5	27±4	At least 3	At least 3	FMD	6
Van Rijn, 2008[32] **(CS)**	Early onset preeclampsia (144)	Normotensive pregnancy (70)	-	-	At least 6	At least 6	sICAM-1	5
			ABSTRACTS					
Christensen, 2015[33] **(CC)**	Preeclampsia (19)	Normotensive pregnancy (19)	-	-	120	120	PWV, AIx, cIMT	1
Lazdam,	Preeclampsia	Normotensive	40	40	72-156	72-156	cIMT,	2

2012[34] **(CS)**	(90)	pregnancy (50)					PWV, AIx	
Murphy, 2014[35] **(CS)**	Preeclampsia (10)	Normotensive pregnancy (40)	32.1±6.1	30.4±4.2	7±0.7	6±0.9	Laser doppler	0

* Results are presented as mean +/-SD or median (IQR) if not specified otherwise

**The sample size for each group represents the number of patients not lost to follow-up

AASI: ambulatory arterial stiffness index; AIx: augmentation index; CC: Cohort; CS: Case-control; cIMT: carotid intima-media thickness; CVD; Cardiovascular disease; FBF: Forearm blood flow; FMD: Flow mediated dilatation; GDM: Gestational diabetes; HDP: Hypertensive Disorders of Pregnancy; miRNA: microRNA; PAT: peripheral arterial tone; PIGF: placental growth factor; PWV: pulse wave velocity; sICAM-1: soluble intercellular adhesion molecule-1; SLE: Systemic lupus erythematous; sVCAM-1: soluble vascular cellular adhesion molecule-1; SEM: standard error of mean; VEGF: vascular endothelial growth factor.

Table S2: Summary of Assessed Modalities

Modalities	Number of studies	Type of Vascular Measurements
Carotid intima-media thickness (cIMT)	16 (10)	Subclinical atherosclerosis (large vessels)
Flow-mediated dilatation (FMD)	17 (13)	Endothelial dysfunction (small vessels)
Pulse wave velocity (PWV)	13 (7)	Arterial stiffness (small vessels)
Augmentation index (AIx)	11 (10)	Arterial stiffness (small vessels)
Laser doppler	4	Endothelial dysfunction (small vessels)
Forearm blood flow (FBF)	5	Endothelial dysfunction (small vessels)
Peripheral arterial tone (PAT)	2	Endothelial dysfunction (small vessels)
Vascular compliance	2	Vascular compliance (large vessels)
Ambulatory stiffness index	1	Arterial stiffness (small vessels)
Large and small artery elasticity index	1	Vascular compliance (large and small vessels)
BIOMARKERS		
Soluble intercellular adhesion molecule (sICAM-1)	11 (5)	Inflammation
Soluble vascular cellular adhesion molecule (sVCAM-1)	10 (5)	Inflammation
Soluble fms-like tyrosine kinase-1 (sFlt-1)	8 (7)	Angiogenesis
Vascular endothelial growth factor (VEGF)	6 (3)	Angiogenesis
Endothelin	4	Thrombosis
Placental growth factor (PIGF)	4	Angiogenesis
Fibronectin	3	Thrombosis
Endoglin	1	Angiogenesis
microRNA	1	Posttranscriptional regulation of gene expression
Growth arrest specific protein 6	1	Angiogenesis
CD33+VEGR1+, CD34+VEGR2+	1	Angiogenesis

Some studies assessed more than 1 modality. Modalities in bold font have been pooled. Numbers in parentheses correspond to the number of studies included in the meta-analysis

Table S3: Absolute Values and Weight Mean Differences Stratified on Median Duration of Follow-up

	Median follow-up (months)	N < median follow-up	Mean value for exposed	Mean value for controls	WMD < median follow-up	N > median follow-up	Mean value for exposed	Mean value for controls	WMD > median follow-up
cIMT (mm)	48	198	0.57	0.54	0·03 [0·01 to 0·05]	604	0.64	0.60	0·02 [-0·02 to 0·07]
AIx (%)	60	173	23.02	11.84	9·92 [5·92 to 13·92]	972	19.92	17.27	2·69 [-1·79 to 7·17]
cfPWV (m/s)	287	854	7.64	7.02	0·63 [-0·16 to 1·42]	233	7.75	7.08	0·54 [0·19 to 0·88]
sFlt-1	94	145	207.1	133.93	10·44 [1·38 to 19·51]	559	140.66	133.13	3·23 [-0·18 to 6·23]

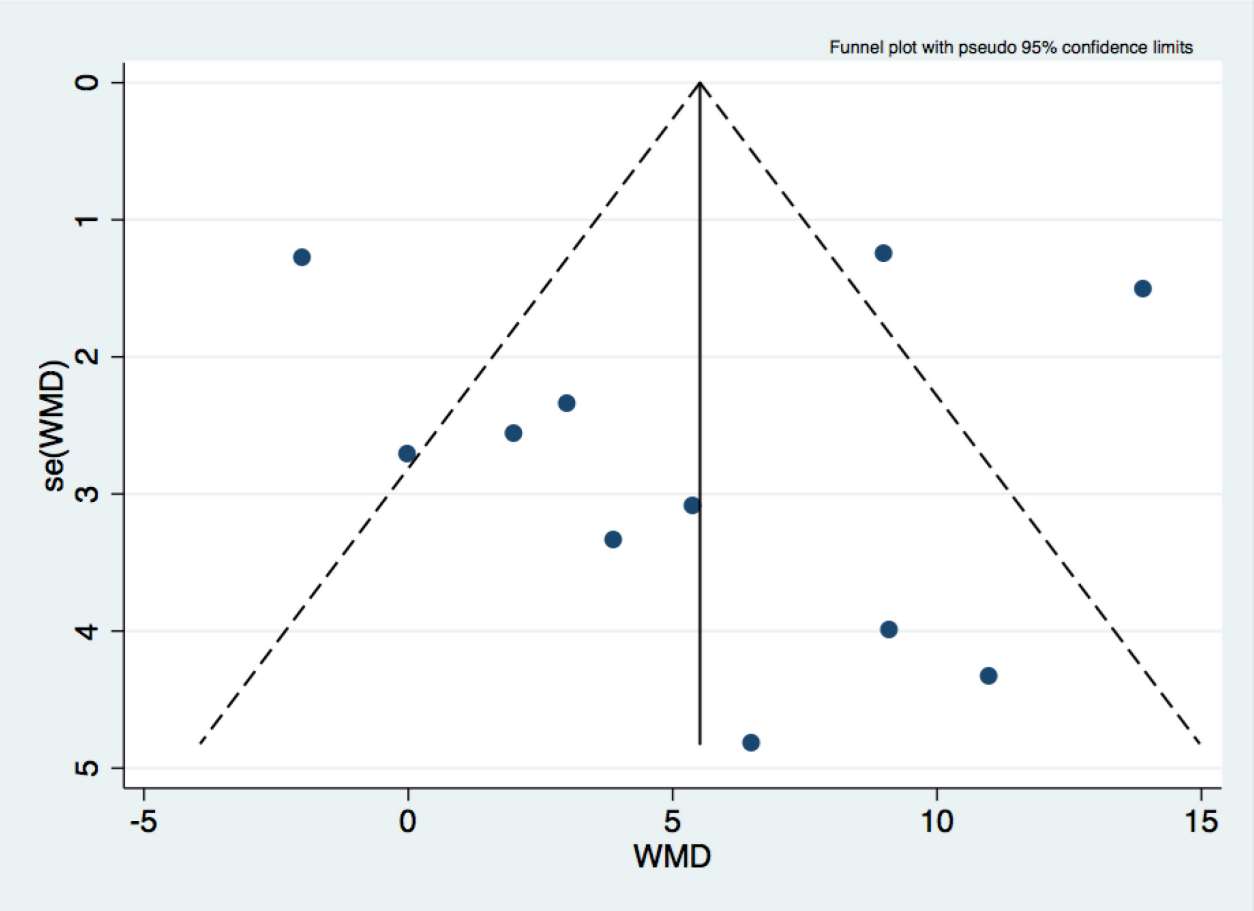

Figure S1: Funnel Plot of Studies Assessing Augmentation Index

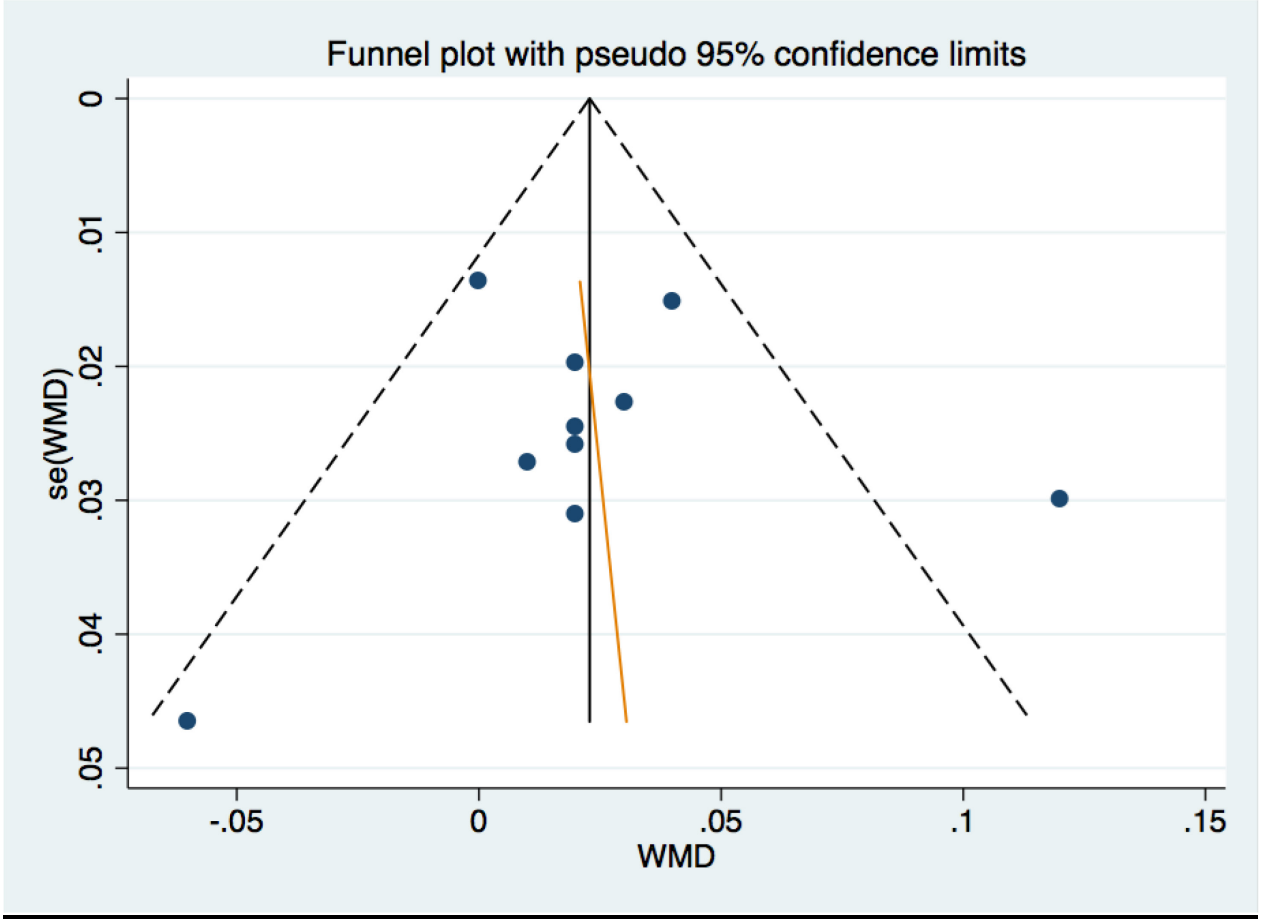

Figure S2: Funnel Plot of Studies Assessing Carotid Intima-Media Thickness