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Preeclampsia - in light of the cardiovascular system

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Abstract

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Preeclampsia affects 2–8% of all pregnancies worldwide. This multi-system disorder, increases the risk of adverse pregnancy outcomes, maternal death, and adverse short- and long-term consequences for the women and infants. Impaired cardiovascular adaptations to pregnancy may contribute to preeclampsia. These women run an elevated risk of future cardiovascular disease (CVD). The mechanisms connecting adverse cardiovascular adaptions, preeclampsia, and CVD remain unclear. To address the rising CVD prevalence in women, identification of sex-specific pathways is needed, particularly in women with prior preeclampsia.

This thesis explores the pathophysiological and predictive role of cardiovascular biomarkers before preeclampsia and preeclampsia´s impact on long-term CVD, using proteomics and register-based data.

In Papers I and II, 92 cardiovascular plasma proteins were analysed. In Paper I, machine learning approach identified Matrix metalloproteinase (MMP)-12 as a novel biomarker for subsequent preeclampsia, including early- and late-onset preeclampsia. In Paper II pathophysiological pathways were explored by comparing cardiovascular proteins in women with subsequent preeclampsia, small for gestational age (SGA) birth, or combined outcomes, with normotensive pregnancies. Only subsequent preeclampsia, was associated with dysregulation of several cardiovascular biomarkers. All outcomes were associated with MMP-12 and placental growth factor (PlGF).

In Paper III, the risk of future CVD in multi-fetal pregnancies complicated by preeclampsia was assessed by adjusted Cox proportional hazard models, comparing them to normotensive singleton pregnancies. Multi-fetal pregnancies complicated by preeclampsia were not associated with increased long-term CVD-risk as observed in singleton pregnancies with preeclampsia.

In Paper IV, a regression model assessed cardiovascular risk factors at the first-time myocardial infarction (MI), comparing women with to without prior pregnancy-induced hypertensive disorders (PIH). Chronic hypertension and elevated body mass index were more prevalent and smoking less prevalent, in those with prior PIH.

In conclusion, analysing cardiovascular biomarkers revealed MMP-12 as a promising predictive cardiovascular biomarker for preeclampsia, and dysregulation of the cardiovascular system specifically in women with subsequent preeclampsia. Women with multi-fetal pregnancies and preeclampsia lack the increased long-term CVD risk observed in corresponding singleton pregnancies, indicating different pathways to preeclampsia. The strong association between chronic hypertension and prior PIH at first MI, indicates its key role in their elevated MI-risk.

Keywords: Biomarker, Prediction, Cardiovascular disease, Chronic hypertension, Pregnancy induced hypertensive disorders, Myocardial infarction, Preeclampsia, Pregnancy, Small for Gestational Age.

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To Ruby, Elsie and Braghe

List of Papers

This thesis is based on the following papers, which are referred to in the text by Roman numerals.

I. **Nordlöf Callbo, P.**, Junus, K., Gabrysch, K., Bergman, L., Sundström Poromaa, I., Lager, S., Wikström, AK., Novel associations between mid-pregnancy cardiovascular biomarkers and preeclampsia; an explorative nested case-control study. *Reproductive Sciences,* 2024 Jan; 31:1391–400.

II. **Nordlöf Callbo P.**, Junus K., Lindberger E., Lindström L., Poromaa I.S., Bergman L., Lager S., Wikström A.K., Cardiovascular Proteins in Preeclampsia and Small-for-Gestational-Age Birth: A Case-Control Study. 2025 May. *Under review*

III. Bergman, L., **Nordlöf Callbo, P.**, Wikström, AK., Snowden, JM., Hesselman, S., Edstedt Bonamy, AK., Sandström, Anna., (2020) Multi-fetal Pregnancy, Preeclampsia and Long-term Cardiovascular disease. *Hypertension*, 2020 June; 76:167–175.

IV. **Nordlöf Callbo P. N.**, Mudrovic, N., Lampa, E., Bergman K., Bergman L., Lager S., Hesselman, S., Christersson C., Junus K., Wikström A.K., Cardiovascular Risk Factors in Women with First-Time Myocardial Infarction and Prior Pregnancy-Induced Hypertension: A Register-Based Cohort Study. 2025 October. Submitted

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Contents

Abbreviations

ACOG	American College of Obstetricians and Gynaecologists
BNP	Brain natriuretic peptide
CD4	T-cell surface glycoprotein
CO	Cardiac output
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
FCGR2B	Low affinity immunoglobulin gamma Fc region receptor II-b
FGR	Fetal growth restriction
FMF	the fetal medicine foundation
FPR	False positive rate
HELLP	Haemolysis, Elevated Liver enzymes, Low platelets
IDUA	Alpha-L-iduronidase
ISOUG	International Society of Ultrasound in Obstetrics and Genecology
ISSHP	The International Society of the study of Hypertension in Pregnancy
IUFD	Intrauterine fetal death
IUGR	Intrauterine growth restriction
LD	Lactate dehydrogenase
LOX-1	Lectin-like oxidized LDL receptor 1
MAP	Mean arterial blood pressure
MMP	Matrix metalloproteinase
NICE	The National Institute for Health and Care Excellence
PAPP-A	Pregnancy Associated Plasma Protein-A
PlGF	Placental growth factor
PIH	Pregnancy induced hypertension
SBP	Systolic blood pressure
SD	Standard deviations
SSOG	The Swedish Society for Obstetrics and Gynaecology
SLE	Systemic lupus erythematosus
SVR	Systemic vascular resistance

Introduction

Preeclampsia, a pregnancy-associated disorder, affects women and infants worldwide, but predominately in low- and middle-resource countries. This is a multisystem disorder, and the third most common cause of maternal mortality, responsible for >70 000 deaths annually (2). It also increases the risk of fetal growth restriction (FGR), neonatal morbidity, and mortality (3).

The pathophysiology behind this disorder remains elusive. However, women at risk of preeclampsia share several traditional risk factors for cardiovascular disease (CVD). Women who have suffered from preeclampsia also have a doubled future risk of CVD compared to those who have normotensive pregnancies (4, 5). To improve both maternal health during pregnancy and women's long-term cardiovascular health, timely prediction, prevention, and treatment of preeclampsia, as well as standardised postpartum follow-up, are warranted (6, 7).

The work (Papers I-IV) reviewed in this thesis aimed to explore mid-pregnancy circulating cardiovascular biomarkers to identify novel predictors for preeclampsia and to gain further knowledge of pathophysiological routes to preeclampsia. Another aim was to study the long-term risk of CVD in women with preeclampsia in multi-fetal pregnancies. Using a registered-based approach, we assessed CVD-risk after preeclampsia, considering plurality of pregnancy. Further using register-based data, we aimed to examine cardiovascular risk factors in women with first-time myocardial infarction (MI) in relation to prior pregnancy-induced hypertension (PIH).

Preeclampsia

Definition

Preeclampsia is a multifaceted disorder, which usually develops during pregnancy, but an early post-partum debut may occur (8, 9). In 2018, the International Society of the Study of Hypertension in Pregnancy (ISSHP) published updated international recommendations for the classification, diagnosis, and management of preeclampsia, which have been further updated (10). Before 2019, the definition recommended by The Swedish Society for Obstetrics and Gynaecology (SSOG) included de novo hypertension and proteinuria. However, after 2019 the definition includes de novo hypertension and 1≥signs of organ engagement (Table 1). Proteinuria is present in the majority (75%) of cases (6, 38). For patients enrolled in scientific research, including those in the Papers (I-IV) reviewed in this thesis, international criteria endorse proteinuria as mandatory to ensure specificity (11, 12).

Table 1. Diagnostic criteria for preeclampsia defined by The Swedish Society for Obstetrics and Gynaecology.

Diagnostic criteria	**Definition**
New-onset hypertension	Systolic blood pressure ≥ 140 mm Hg and/ or diastolic blood pressure ≥ 90 mm Hg on two occasions at least 4 hours apart after 20 weeks of gestation in a woman with a previously normal blood pressure
One or more of the following new onset conditions at or after 20 weeks' gestation.	
Renal insufficiency	Significant proteinuria (verified by a randomly taken albumin/creatinine ratio ≥ 8 mg/mmol or U-protein/albumin ≥0.3g/24h.)
Impaired liver function	Elevated blood concentrations of liver transaminases to twice normal concentration. Epigastric pain. Pain beneath the right arcus.
Haematological effects	Decreasing number of platelets. Thrombocytopenia (platelet count <150 000/µL). Haemolysis (haptoglobin <0.25 g/L or Lactate dehydrogenase* 600 U/L or >10.0 $ukat$/L).
Neurological effects	Severe headache. Persistent visual symptoms. Plantar clonus, seizures (eclampsia). Stroke.
Circulatory effects	Pulmonary oedema. Chest pain
Uteroplacental dysfunction	Fetal growth restriction

Subtypes of preeclampsia

Preeclampsia is suggested to results from several distinct pathophysiological pathways converging into shared clinical symptoms. Together with gestational hypertension, they are commonly referred to as pregnancy-induced hypertensive (PIH) disorders (13). In clinical practice, categorising preeclampsia as mild or severe is misleading; instead, describing it as with or without severe features is preferred (8). Given its potential for rapid progression to a life-threatening condition, preeclampsia should be regarded as a single clinical entity in practice (8).

In order to address differences in pathophysiology, symptoms, and severity of preeclampsia, it is categorised into different subtypes (8, 14). In scientific research, the distinction between early- and late-onset (</≥34+0 weeks of gestation) preeclampsia is currently recommended by the ISSHP (8, 14). Early-onset preeclampsia confers a higher risk for adverse maternal and fetal outcomes, complicating 0.4% of pregnancies (3). This phenotype often presents with FGR, fetal and perinatal death, and severe fetal morbidity (3, 15). Late-onset preeclampsia, however, complicates 2.7% of pregnancies and accounts for the majority of preeclampsia cases in high-resource settings (3, 16). In research, as the exact onset of preeclampsia is rarely distinct and sample sizes are often limited, gestational age at birth, categorised as preterm (<37 weeks) or term (≥37 weeks), is commonly used as a proxy (17, 18). In Sweden, about 20% of women with preeclampsia deliver preterm (19).

Severe features of preeclampsia include systolic blood pressure (SBP)≥160 mmHg and/or diastolic blood pressure (DBP) ≥110 mmHg, significant organ dysfunction, severe FGR or preterm birth. Severe features also include preeclampsia manifested as Haemolysis, Elevated Liver enzymes, Low platelets (HELLP) syndrome or eclampsia during pregnancy, intrapartum or postpartum, none of which require hypertension or proteinuria for diagnosis (10). The HELLP syndrome is associated with elevated rates of maternal morbidity and mortality (8, 9). Further, eclampsia is described as generalised tonic-clonic seizures, not explained by other causes. In women with eclampsia 16% are normotensive before seizures (8, 9).

Epidemiology and risk factors

Worldwide, preeclampsia affects 2–8% of pregnancies, with incidence rates being two- to threefold in low- and middle-resource countries compared with high-resource countries (20). In the United States, Canada, and Western Europe rates are 2–5%. However, in low-resource settings, incidence and maternal mortality rates due to preeclampsia are higher (21). In multi-fetal pregnancies, the incidence of preeclampsia is two- to fourfold than in singleton pregnancies. Preeclampsia also accounts for around 500 000 fetal and neonatal deaths (22).

Identification of women in early pregnancy at high risk of developing preeclampsia remains key to improving maternal and infant health. Epidemiological studies have identified several risk factors for preeclampsia, many of which are shared with CVD (Table 2) (10, 23). Some risk factors, e.g., chronic hypertension and African American origin, confer a higher risk for early-onset preeclampsia, while diabetes mellitus, nulliparity, and young age are strongly associated with late-onset preeclampsia (9, 24).

Table 2. Clinical risk factors for preeclampsia as stated by the Swedish Society for Obstetrics and Gynaecology.

Clinical risk factors for preeclampsia	
Moderate risk	***High risk***
Family history of preeclampsia	Auto immune disease (SLE or APS)
African origin	Previous preeclampsia or eclampsia
Verified obstructive sleep apnoea	Previous gestational hypertension with birth < 34 weeks or IUGR or IUFD or placental abruption
"White coat hypertension"	Diabetes type 1 or 2
Nulliparity	Multifetal pregnancy
Obesity (body mass index>30)	Chronic kidney disease
Age > 40	Chronic hypertension
Chronic hypertension	In vitro fertilisation with oocyte donation
Pregnancy interval > 10 years	
SBP>130 or DBP > 80 at admission	

SBP indicates systolic blood pressure; DBP, diastolic blood pressure; SLE, systemic lupus erythematosus; APS, anti-phospholipid syndrome; IUGR, intrauterine growth restriction; IUFD, intrauterine fetal death.

Cardiovascular changes in normal pregnancy

In normotensive pregnancies, the maternal cardiovascular system undergoes major adaptations to meet the increased physiological demands required to support both maternal and fetal health. These include vascular adaptions, neurohormonal response, volume expansion, and cardiac remodelling.

Vasodilation and reduced systemic vascular resistance (SVR), driven by increased oestrogen, progesterone, and relaxin, reach their nadir in the second trimester, before they increases in the third trimester, and return close to baseline postpartum (25). While SBP remains relatively stable, DBP decreases during the first- and second trimesters (26-28).

To compensate, sympathetic and renin-angiotensin-aldosterone system (RAAS) activity increases during the first trimester, promoting sodium and water retention, expanding plasma volume by about 45% (25, 29). This causes eccentric cardiac remodelling during the first trimester, with a 50% increase in the left ventricular mass (30). During the first and second trimester, increased stroke volume (SV) and heart rate (HR) will result in an at least 30% increase in cardiac output (CO) (27, 31). Notably, with advancing maternal age, CO tends to decrease and SVR increases in uncomplicated singleton pregnancies, highlighting the increased cardiovascular risk in older mothers through an inverse relationship between cardiac function and age (32).

In multi-fetal pregnancies, the cardiovascular system is put under additional stress due to a higher circulating blood volume. In these pregnancies CO is increased and peripheral vascular resistance is reduced in comparison to singleton pregnancies (33).

The demands placed on the cardiovascular system during pregnancy are often considered an early stress test for latent CVD (34). Failure to achieve these adaptations is associated with PIH and FGR, particularly in cases of preeclampsia (23, 31).

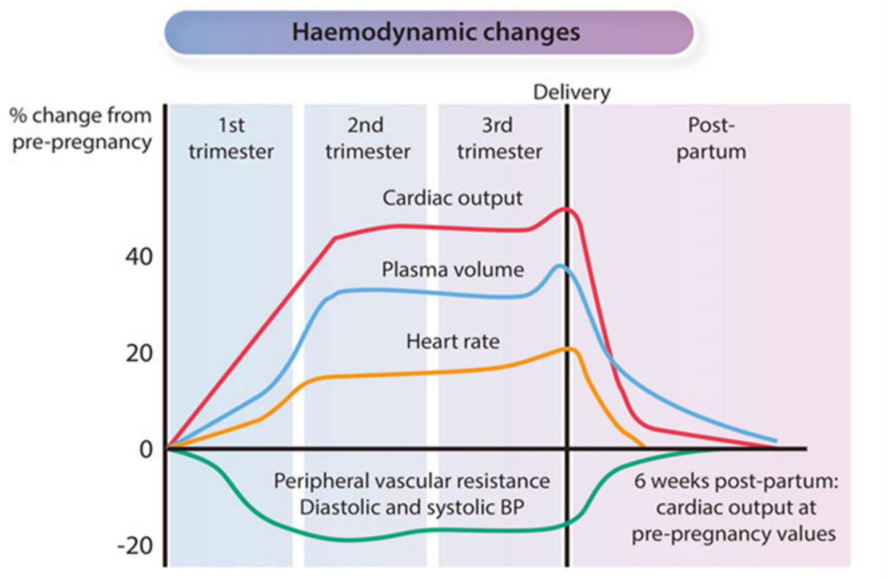

Figure 1. Physiology of haemodynamic changes during and post pregnancy. BP indicates blood pressure. Originally published in ESC Guidelines for the management of cardiovascular disease and pregnancy, European Heart Journal, Oxford Academic, 2025. Republished with permission from Oxford Academic.

Pathophysiology of preeclampsia

The role of the placenta in preeclampsia

Despite extensive research, the pathophysiology behind preeclampsia remains poorly understood (13, 35). During the last decades, the role of the placenta has been central in this context (Figure 2) (13, 17).

The placenta is a multifunctional organ essential for maternal and fetal health. Around 6–7 days post-fertilisation, the blastocyst implants and trophoblast cells invade the endometrium, eventually developing tertiary villi with fetal arteries within their core (36). These cells are also essential for uterine spiral artery remodelling, enabling vascular dilation and a low-resistance placental blood flow. During the first trimester, partial occlusion of spiral arteries, limits access to oxygen, protecting the embryo from oxidative stress (37).

In 1991, Redman et al. described that poor placentation and placental ischemia, is associated with preeclampsia, preterm birth, and FGR (38, 39). This model has been further refined to describe two main pathophysiological stages: (1) poor trophoblast invasion and inadequate remodelling of the uterine arteries, and (2) subsequent placental ischaemia. This results in syncytiotrophoblast stress and dysfunction, resulting in the increased release of anti-angiogenic factors [soluble fms-like tyrosine kinase-1 (sFlt1) and soluble endoglin (sEng)) and decreased release of pro-angiogenic factors (placental growth factor (PlGF)] from syncytiotrophoblasts (17, 40, 41) into the maternal circulation. Additionally, syncytiotrophoblast stress activates the maternal immune system, triggering an inflammatory response, with elevated proinflammatory cytokines (e.g., tumour necrosis factor-α, interleukin (IL)-6) and reduced anti-inflammatory cytokines (e.g., IL-10, IL-4) (42, 43). The dysregulation of these factors may target maternal endothelium and trigger its dysfunction, leading to organ failure (13, 17, 44).

In multi-fetal pregnancies, preeclampsia could result from placental outgrowth relative to uterine capacity rather than adverse placentation. This could cause compression of the terminal villi causing disrupted placental perfusion and hypoxia with further maternal endothelial damage due to the release of antiangiogenic factors (9, 26).

The development of preeclampsia involves different complex pathways to placental hypoxia, as supported by histological findings. Placentas from early-onset or preterm preeclampsia are typically smaller and show signs of poor perfusion, infarctions, and atherosclerosis-like lesions, which are less common in late-onset preeclampsia (39, 45). Although no strict gestational age separates these pathways, early-onset preeclampsia is more often associated with impaired placental development. In late-onset preeclampsia, however, placental hypoxia is hypothesised to be secondary to overcrowding of villi. The second stage may theoretically last for several months in early-onset preeclampsia, but only a few weeks in late-onset preeclampsia (38, 39).

Furthermore, maternal proinflammatory conditions, i.e., diabetes, hypertension, obesity, and autoimmune disorders, may impair the tolerance to angiogenic and inflammatory imbalance. Combined with placental factors, the inflammatory and angiogenic imbalance may become overwhelming, leading to manifest preeclampsia (13).

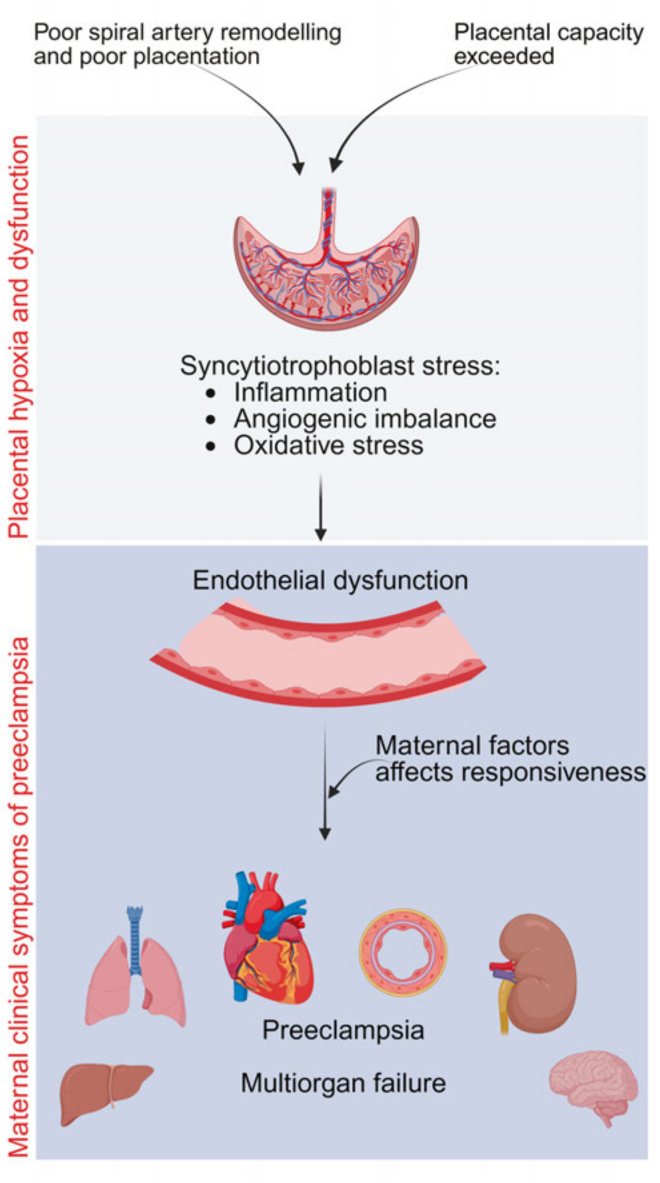

Figure 2. The placenta and pathways to preeclampsia. Maternal factors indicate predisposing conditions such as obesity, chronic hypertension and autoimmune diseases. Created in Biorender.com.

Cardiovascular changes in preeclampsia

Women with preeclampsia have abnormal cardiovascular adaptions to pregnancy before clinical symptoms (46, 47). Already before pregnancy, these women have higher resistance circulation, higher blood pressure, and altered CO (48). Increased arterial stiffness is also common, possibly reflecting a pre-existing fragile vasculature (49).

Although these women may have a greater drop in blood pressure during the first trimester, it increases again late in the first trimester and continues as the pregnancy proceeds (48-50). Postpartum, the fall in blood pressure is less than in healthy pregnancies (48). These mal-adaptations may be partly driven by dysregulated expression of vasoactive proteins, including angiotensin II, endothelin-1, and thromboxane A2, which contribute to increased vasoconstriction and elevated mean arterial pressure (50).

Furthermore, as opposed to normotensive pregnancies, women that develop preeclampsia show concentric cardiac remodelling (thickening of the ventricular wall without an increase in chamber size) and diastolic dysfunction, secondary to the increased SVR and afterload (23, 46, 51). Although some degree of cardiac dysfunction may occur in normotensive pregnancies, they return to baseline postpartum. However, in women with preeclampsia, adverse cardiac remodelling may persist, increasing their risk of developing hypertension within the next few years (28, 52).

As adverse cardiovascular adaptions may occur before placental development is complete, it has been suggested that cardiovascular imbalance may affect placentation (48). However, the impact of these mal-adaptions may differ depending on the phenotype of preeclampsia. Women who develop early-onset preeclampsia or FGR, often have a high vascular resistance and decreased CO through-out pregnancy. Whereas in women with late-onset preeclampsia low vascular resistance and high CO have been observed (47). Conversely, woman with late-onset preeclampsia, have low CO, normal or low vascular resistance, and an intravascular fluid overload (47).

Previous studies have shown that these adverse cardiovascular changes are supported by dysregulation of cardiovascular biomarkers reflecting cardiovascular dysfunction, endothelial injury and generalised oxidative stress (50). Cardiovascular biomarkers are not only dysregulated during these pregnancies, but also long after giving birth, and associated with adverse cardiovascular events later in life (53).

Endothelial dysfunction in preeclampsia

Endothelial dysfunction plays a central role in the clinical symptoms of preeclampsia. In affected women, the maternal vascular endothelium is activated due to increased production and circulation of antiangiogenic proteins.

Currently, some key proteins of the angiogenic imbalance and subsequent endothelial dysfunction have been identified. These include soluble variant of vascular endothelial growth factor (VEGF) receptor-1 (sFlt-1), which binds and inhibits VEGF, and PlGF. PlGF is a glycoprotein which belongs to the vascular VEGF-family. It is predominantly expressed in the syncytiotrophoblast layer of the placenta, although it is also found in other tissues. During pregnancy, PlGF plays a crucial role in the development of the placental vasculature. Its levels begin to rise toward the end of the first trimester, peaking at around 30 weeks of gestation. After this peak, PlGF levels decline while sFlt-1 levels increase.

Decreased levels of PlGF, compared to normotensive controls, are associated with both preeclampsia and FGR. This pattern is suggested to be mediated by excess placental sFlt-1 production in women who develop preeclampsia. However, it has been suggested that in pregnancies with FGR, a modest increase in sFlt1 and a decreased production of PlGF contribute to the total decrease in PlGF (54-56). In normal pregnancies, sFlt-1 levels increase at 33 to 36 gestational weeks with following decrease in PlGF levels. This shift from a proangiogenic to a more antiangiogenic state is observed earlier in women who develop preeclampsia, and a greater shift is particularly associated with early-onset preeclampsia and preeclampsia with FGR (42, 57).

Prediction of preeclampsia

> *"Screening is the presumptive identification of unrecognized disease or defect by the application of tests, examinations, or other procedures which can be applied rapidly. Screening tests sort out apparently well persons who probably have a disease from those who probably do not. A screening test is not intended to be diagnostic."* Wilson and Junger (1)

In 1968, Wilson and Junger, outlined 10 principles for use by the World Health Organization to assess whether screening is appropriate for various public health issues (58). Given that preeclampsia is a global and growing health concern with adverse effects on maternal and infant health, first-trimester screening is ideal to enable timely prevention with low-dose aspirin. International guidelines currently recommend first-trimester screening either by assessing maternal risk factors or The Fetal Medicine Foundation (FMF) algorithm (Table 3).

Table 3. First-trimester screening recommendations by international committees

	Prophylaxis
Screening by Maternal risk factors	
ACOG	81 mg daily aspirin prophylaxis if >1 moderate or ≥1 high-risk factor risk factor (59) and discontinued at birth (60).
NICE	75-150 mg daily aspirin prophylaxis if >1 moderate or ≥1 high-risk factor risk factor (61) and discontinued at birth (61).
SSOG	75 mg daily aspirin prophylaxis if ≥3 moderate or ≥1 high-risk factors (62) and discontinued by 36 gestational weeks (62).
Screening by the Fetal Medicine Foundation algorithm	
ISSHP	150 mg daily aspirin prophylaxis to high-risk women, and discontinued by 36 weeks gestation.
FIGO	150 mg daily aspirin prophylaxis in high-risk women and discontinued by 36 weeks gestation

ACOG; The American College of Obstetricians and Gynecologists, NICE; National Institute for Health and Care Excellence, SSOG; ISSHP; International Society for the Study of Hypertension in Pregnancy, FIGO; The International Federation of Gynecology and Obstetrics.

The association between maternal risk factors and preeclampsia has been studied extensively (24). Both the National Institute for Health and Care Excellence (NICE) and The American College of Obstetricians and Gynecologists (ACOG) guidelines, treat each maternal risk factor as an independent screening criterion, resulting in additive detection and screen-positive rates (63). However, the risk factors differ between the guidelines and their overall performance is limited. The NICE method achieves a detection rate of 35% for all preeclampsia cases, with 39 % for preterm and 34% for term preeclampsia (10% false-positive rate). In contrast, ACOG's approach shows higher detection rates, 90% for preterm and 89% for term preeclampsia, but at the cost of a significantly higher false-positive rate (FPR) of 64% (63). In conclusion, screening based solely on maternal risk factors remains suboptimal, highlighting the need for more efficient models.

The FMF algorithm, uses a competing risks approach (Bayes' theorem) estimating a patient-specific risk of preeclampsia being requiring birth before a specified gestational age. It combines maternal risk factors with various combinations of biophysical (uterine artery Doppler pulsatility index, mean arterial blood pressure), and biochemical [plasma PlGF and Pregnancy-Associated Plasma Protein A (PAPP-A)] measurements in the first trimester. The method is higher detection rates than using only maternal risk factors (63, 64). This algorithm has been prospectively evaluated in different populations yielding varying detection rates, 64–100% for early-onset, 75–82% for preterm, and 41–47% for term preeclampsia (FPR 10%) (65).

To focus resources on women at high risk of preeclampsia, a two-stage prediction model has been proposed: (1) Primary screen with maternal factors alone or together with mean arterial blood pressure (MAP) and uterine artery pulsatility index (UtA-PI), (2) Screening with PlGF or the FMF algorithm for those who test positive in the previous step. These two-step models achieve detection rates similar to those obtained by screening all pregnant women with the second step, and it is also financially beneficial (65, 66). However, prediction of term preeclampsia remains poor when using the FMF algorithm in the second trimester (67).

Currently a different approach is risk assessment of term preeclampsia using the FMF algorithm at 35-36 weeks gestation to identify women at high risk. Term preeclampsia and following adverse outcomes, may than be prevented by timed birth (68).

Although preterm preeclampsia may present with more severe symptoms, term cases are more common and can lead to serious complications, making these women an important screening target (65, 69). Current prediction models for late-onset and term preeclampsia remains moderate (70, 71). Since current biomarkers predominantly reflect placental dysfunction, which may dominate pathways to early-onset disease, new biomarkers are needed to improve first- and second trimester prediction of late-onset preeclampsia (65, 67, 72).

The cardiovascular system in preeclampsia is receiving increasing attention (23). While cardiovascular biomarkers have primarily been studied in women with overt preeclampsia, knowledge on their predictive potential remains limited (73-75). Recent advancements in proteomics allow high-throughput detection and mapping of numerous proteins without loss of sensitivity. These techniques serve as hypothesis-generating tools that hold promise for discovering biomarkers expressed before preeclampsia onset and may improve prediction models (76, 77).

Prevention of preeclampsia

There is an international consensus that early detection of mothers at risk of preeclampsia enables preventive measures. No current treatment can prevent preeclampsia in all women, but some approaches may reduce the risk. In addition, recognising women at high-risk improves individualised assessment of risks and healthcare surveillance for timely detection, whereas delayed diagnosis contributes significantly to maternal morbidity and mortality (78).

Over the past decades, various preventative strategies to reduce preeclampsia have been evaluated (16). Women at risk of preeclampsia should receive counselling on lifestyle and dietary modifications. Regular physical activity may lower the risk of preeclampsia and other PIH (79, 80). Calcium supplements before pregnancy have previously been suggested to the reduce the risk of preeclampsia in women with low dietary calcium intake. However, a recent review suggests that these supplements may have little to no difference (81).

The ASPRE trial published in 2017, using the FMF-algorithm, found a 62% reduction of preterm preeclampsia among high-risk women taking 150 mg aspirin compared to placebo (82). In another population using the same algorithm, the reduction was 41% (83). Currently, aspirin prophylaxis at 75-150 mg is considered the most effective intervention for preventing preterm preeclampsia, but it does not reduce the risk of term preeclampsia, likely due to a different pathophysiology than that of preterm cases (70). However, optimal dosage of aspirin is debated, balancing the risk of insufficient prophylaxis at lower doses against potential complications at higher doses. A cohort study showed that aspirin use increases the risk of postpartum haemorrhage by 20% (84). Previous randomised controlled trials indicate that a daily dose of 150–162 mg gives a more effective risk-reduction than 75–81 mg. However, these studies are small sizes and some have a high risk of bias (85). In a Swedish cohort study that included over 13 000 women, use of low-dose aspirin (75mg) compared to use of high-dose aspirin, showed no altered risk of preeclampsia or preterm preeclampsia in high-risk women (86).

Current guidelines recommend initiating aspirin prophylaxis before 16 weeks of gestation in high-risk women, continuing until diagnosis of preeclampsia, gestational week 36, or birth (34, 60-62). Despite some conflicting evidence, initiation of low-dose aspirin before 28 gestational weeks may also improve pregnancy outcomes (9, 10, 87-89).

In Sweden, SSOG recommends aspirin prophylaxis at 75 mg daily for women with at least three moderate risk factors or one high risk factor (Tables 2 and 3) (62). This recommendation aligns with international standards. However, both NICE and ACOG, have a more inclusive approach, advising initiation of aspirin prophylaxis when >1 moderate risk factor is present (9, 63).

Clinical aspects of preeclampsia

The onset of preeclampsia is often insidious, with varying symptoms. Independent of the severity of primary symptoms, it may rapidly become life-threatening for the mother and fetus. Despite excessive research, giving birth remains the only current cure (10, 90).

The SSOG recommend that all pregnant women should be monitored regarding their blood pressure at each antenatal visit. In the presence of maternal risk factors for preeclampsia or debut of elevated blood pressure or symptoms, proteinuria is screened for (62).

Clinical management of overt preeclampsia is mainly symptomatic and focused on preventing maternal morbidity and mortality (91). Guidelines from international societies, i.e., ACOG, FIGO, NICE and ISSHP are continuously updated. The main recommendations are similar but may differ in clinical practices between countries. The general recommendations for preeclampsia management include clinical assessment of mother and fetus. Treatment and observation of blood pressure should be initiated and continued through birth and the antenatal period as the blood pressure may fluctuate (10, 12, 62). In the presence of severe features and/or eclampsia, parenteral administration of magnesium sulphate is recommended (10, 12, 62).

Although giving birth is the only definite treatment for preeclampsia, it must balance the maternal and fetal risks (9). SSOG recommends expectant management <37 gestational weeks if no severe features are present, but after 37 gestational weeks birth is recommended. For severe preeclampsia or if unstable maternal or fetal conditions independent of gestational age, birth is recommended once the women's condition is stabilised (35).

Postpartum blood pressure and other clinical parameters should be monitored in hospital for at least 72h. Furthermore, antihypertensive medication should be continued and adjusted, and thromboprophylaxis during the first week postpartum should be advised in those with a caesarean section (62).

On discharge, a follow-up plan including weekly blood pressure measurements and reassessment of antihypertensive therapy should be conducted. Similar to ISSHP, the SSOG advises that all women with PIH be offered a follow-up meeting with their obstetrician 8–12 weeks postpartum for evaluation of blood pressure, urine- and blood samples, neurological symptom, and counselling on future risk of PIH and CVD. For all women with PIH, annual check-ups for blood pressure and other cardiovascular risk factors are recommended. In women with preeclampsia including severe features, cardiovascular risk assessment by a general practitioner should be conducted on a yearly basis (10, 62).

The ISSHP also endorses home blood pressure monitoring, which is well studied in the non-pregnant hypertensive population, as a valuable tool for diagnosing white coat hypertension, masked hypertension, and transient gestational hypertension (92, 93). In pregnant with PIH, home blood pressure monitoring reduces healthcare visits, preeclampsia diagnoses, and labour inductions, possibly due to fewer cases of clinically significant hypertension (94, 95). Moreover, postpartum home blood pressure measurement combined with self-managed antihypertensive therapy lowered DBP compared to controls, and this difference sustained over 3-4 years postpartum (96-98).

Table 4. Postpartum follow up after preeclampsia by international committees.

Society	Postpartum
	Within one-two weeks after discharge from hospital
ACOG	• Blood pressure check at obstetric provider.
FIGO	• Initiate targeted interventions to improve cardiovascular risk.
ISSHP	• Reviewed within 1 week if still on anti-hypertensives.
NICE	• Check blood pressure at 3-5 days postpartum. • Follow-up plan of blood-pressure. • Medical review after 2 weeks on antihypertensive treatment.
SSOG	• Check blood pressure at day 5-7. • If antihypertensive treatment persists, follow-up at physician <2 weeks.
	6-12 weeks
ACOG	• Check blood pressure 6 weeks postpartum at primary care.
FIGO	• Within 6–12 weeks, follow-up with multidisciplinary team approach assessing cardiovascular risk factors.
ISSHP	• Check cardiovascular risk factors after 12 weeks. • If persistent proteinuria or hypertension, referral to general practitioner.
NICE	• A medical review within 6–8 weeks with their general practitioner or specialist. If proteinuria, assess kidney function at 3 months postpartum.
SSOG	• Within 8–12 weeks: Follow-up meeting with a physician assessing cardiovascular risk factors and neurological symptom, and counselling on future risk of PIH and CVD. • If persistent hypertension, proteinuria or abnormal blood samples results referral to appropriate unit.
	6 months
FIGO	• Each 6–12 months: check blood pressure and introduce treatment if needed. • Cardiovascular and metabolic risk screening within 1 year.
ISSHP	• Check blood pressure, if≥ 120/80 mmHg discuss lifestyle changes.
NICE	• Consultation on cardiovascular risk reduction with general practitioner or specialist.
	Annually
ACOG	• If preterm pre-eclampsia or recurrent pre-eclampsia assess cardiovascular risk factors.
FIGO	• Cardiovascular screening every 4–6 years.
ISSHP	• Calculate life-time cardiovascular risk scores. • Check cardiovascular risk factors. • Annual medical review following 5–10 years. • Healthy lifestyle and blood pressure <120/80 mmHg.
SSOG	• Check blood pressure and cardiovascular risk factors.

ACOG; The American College of Obstetricians and Gynecologists, NICE; National Institute for Health and Care Excellence, SSOG; ISSHP; International Society for the Study of Hypertension in Pregnancy, FIGO; The International Federation of Gynecology and Obstetrics. ESC; European Society of Cardiology

Long-term cardiovascular outcomes after preeclampsia

CVD is a global health issue, and it is the leading cause of death in women accounting for approximately one-third of total deaths. Alarmingly, a rise in MI in younger women has been documented (99, 100). Furthermore, excess mortality due to coronary artery disease is more predominant in women than in men (101, 102).

CVD in women in general is driven by established cardiovascular risk factors e.g., hypertension, dyslipidaemia, diabetes, obesity, and smoking. Hypertension is the leading risk factor, and it has a higher association to MI and stroke in women than it does in men. Moreover, obesity, which is the primary modifiable risk factor for hypertension, is highly prevalent and increasing globally. Despite improved awareness, sex-specific symptoms and risk factors in women remain poorly understood, underestimating women's cardiovascular risk (103, 104).

Due to pregnancy's demands on the cardiovascular system, it is regarded a natural stress test for women's cardiovascular system, providing valuable insight into future cardiovascular health (23). In addition, repeated large-population studies show that preeclampsia shares common risk factors for CVD, which may be present years before pregnancy, indicating latent CVD (Table 2) (105). After PIH, women tend to have elevated cardiovascular risk factors that remain elevated even beyond 50 years of age. Although cardiovascular risk factors progress in all women with age, those with a history of PIH show a greater increase in SBP and adiposity (106).

Women previously experiencing preeclampsia or other PIH run a doubled risk of early cardiac or peripheral arterial disease and premature cardiovascular mortality (107, 108). The risk is enhanced for preeclampsia with severe features and recurrent preeclampsia, both having additive associations to fatal or nonfatal ischaemic heart disease (107-109). Already 3 months postpartum women with prior preeclampsia, have a high prevalence of persisting hypertension, which may remain during the following years (110-112).

Cardiovascular changes in those who develop preeclampsia are similar to those in non-pregnant women who later develop CVD (23). In a pregnancy complicated by preeclampsia, the hemodynamic changes, such as asymptomatic left ventricle hypertrophy, systolic and diastolic dysfunction, and prehypertensive state, persist at 1 year postpartum (23). These indications of myocardial damage are particularly common in women with previous preterm preeclampsia as opposed to term (23, 112-114). Such changes support the theory of the long-term cardiovascular legacy of preeclampsia (23).

It is remains unclear whether shared risk factors cause both preeclampsia during pregnancy and later CVD in the same women. A previous study observed that after adjusting for pre-pregnancy risk factors, women with PIH still showed increased CVD risk, supporting that the elevated risk of CVD lies beyond shared pre-pregnancy risk factors and that this risk also may be pregnancy mediated (115-118). Theoretically, preeclampsia may permanently compromise the maternal cardiovascular system, introducing both a *de novo* risk but also increasing a current CVD-risk (23, 108).

In summary, preeclampsia can be viewed as a cardiovascular stress test induced by pregnancy, unmasking women at increased risk of future CVD. Regardless of the underlying mechanisms, pregnancy provides an early opportunity to identify, monitor, and address cardiovascular risk factors (Figure 3).

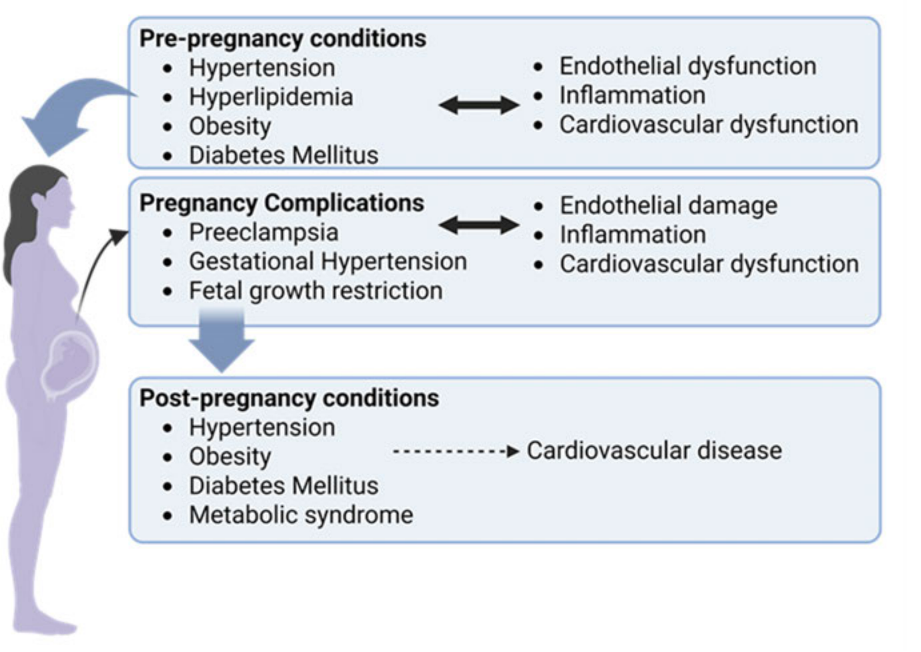

Figure 3. Associations between pre-pregnancy risk factors, adverse pregnancy outcomes, and post-pregnancy cardiovascular risk factors. Created in BioRender.com.

Fetal growth restriction

Definition

Fetal growth restriction (FGR) implies that the fetus has a pathological restriction of its hypothetical genetic growth potential (119, 120). Although there is no universally accepted threshold, FGR defined by an estimated fetal weight or abdominal circumference below the 3rd or 10th percentile, often accompanied by abnormal uterine artery pulsatility index (121).

Commonly the statistical term, small for gestational age (SGA), is used to describe a fetal weight or birth weight that is below a specific threshold of average weight for gestational age (119). However, the definition of SGA is not synonymous with FGR. In contrast to FGR, an SGA infant may be healthy but constitutionally small (119, 120). However, the majority of SGA infants are subjected to FGR, and infants exposed to FGR are commonly SGA (122).

Furthermore, FGR may be categorised into early-onset (<32 gestational weeks) and late-onset (≥32 gestational weeks) FGR. Early-onset, in contrast to late onset FGR, is associated with preeclampsia, abnormal umbilical artery Doppler findings and adverse perinatal outcomes. In addition, early-onset FGR is also associated with an angiogenic imbalance and adverse placentation. Differentiation between these subtypes may imporve management (123).

Epidemiology

Impaired fetal growth appears in about 3-5% of all pregnancies (122). Preeclampsia, increases the incidence of FGR by two- to threefold (119).

Growth restricted fetuses are more commonly delivered premature, due to maternal or fetal health concerns. This may further increase their risk of adverse short- and long-term outcomes. A growth restricted fetus is more prone to perinatal morbidity and mortality, including adverse perinatal cerebral damage (119-122). In addition they have a higher incidence of short- and long-term neurological sequalae as well as cardiovascular and endocrine disorders in adulthood (119). Some of these outcomes may be secondary to premature birth, while others are associated with the impaired fetal growth. Awareness of SGA before birth and a structured surveillance of fetuses identified as SGA significantly lowers the risk of adverse fetal outcomes (122). Therefore, identification of SGA infants before birth is a priority for obstetric units (122).

Pathophysiology

A balanced interaction between the maternal and fetal circulation in the placenta is essential for a fetal-friendly environment with a sufficient exchange of nutrients and oxygen. FGR originates from complex genetic and environmental mechanisms consisting of 1) fetal factors: chromosomal abnormalities, genetic syndromes, intrauterine infections and multiple gestation pregnancies, 2) maternal factors: hypertensive and metabolic disorders, malnutrition, advanced maternal age, previous stillbirth, autoimmune disorders, and smoking, 3) utero-placental factors: defective placentation (124-126).

Overlapping origins to FGR may contribute to a non-profitable utero-placental circulation and fetal nutrition. Due to abnormal placental development, placentas of growth-restricted infants are smaller and show restricted trophoblast invasion and remodelling of spiral arteries, as well as smaller exchange surfaces of peripheral villi, and fetal capillaries. Consequently, utero-placental vascular resistance is increased, with resultant compromised utero-placental perfusion (124, 127). These changes have been observed in both isolated FGR and when FGR is combined with preeclampsia (124).

Furthermore, women with FGR have different cardiovascular profile during pregnancy compared to women without FGR pregnancies. This profile is characterised by high peripheral vascular resistance and low CO compared to controls and is associated with abnormal changes in the uterine artery (47, 128).

Shared and separate pathophysiology of preeclampsia and fetal growth restriction

Preeclampsia and FGR have overlapping pathophysiology. Both conditions are associated with placental dysfunction (129), maternal cardiovascular dysfunction (5, 47, 48, 130-133). Despite extensive research it remains unclear why some women develop one or both conditions.

Shared maternal risk factors for these disorders include conditions with endothelial dysfunction and elevated inflammation i.e., obesity, insulin resistance, dyslipidaemia and chronic hypertension – all established predictors for cardiovascular disease (5, 133-135). In both preeclampsia and FGR, although to varying degrees, angiogenic biomarkers are deranged, and uterine artery doppler results are affected, suggesting that endothelial dysfunction may precede clinical onset (128, 133).

Comparative studies suggest that women with preeclampsia to a greater extent than in those with FGR, exhibit inflammatory and metabolic imbalance early in pregnancy (133-135). These differences reflect separate pre-pregnancy maternal health, which is proposed to affect the development of preeclampsia and/or FGR. It has been proposed that in preeclampsia, impaired placentation combined with maternal predisposition leads to overt maternal symptoms. In FGR similar placental pathology may occur without maternal manifestations due to a more resistant maternal metabolic and inflammatory profile (133, 136).

In addition, women with FGR show an adverse cardiovascular adaptation to pregnancy similar to that seen in early-onset preeclampsia (48).

Comparable to preeclampsia, women who experience FGR during pregnancy face a nearly doubled long-term risk for CVD compared to those with uncomplicated pregnancies (109, 137).

To summarize, FGR and preeclampsia have both shared and different maternal and placental profiles, indicating complex pathways leading to these disorders. Identifying both shared and unique mechanisms could improve prevention, detection, and management of these closely related disorders.

Aims

The overall aim of this thesis was to gain deeper knowledge of the relation between preeclampsia and the cardiovascular system, with focus on biomarkers and long-term cardiovascular outcomes.

Specific aims were:

I. To explore potentially predictive biomarkers for preeclampsia, by analysing associations between 92 circulating mid-pregnancy cardiovascular proteins and subsequent onset of preeclampsia, distinguishing between early- and late-onset preeclampsia.

II. To explore distinct and shared pathways in subsequent preeclampsia and SGA birth compared to normotensive pregnancies by investigating associations with 92 circulating mid-pregnancy cardiovascular proteins.

III. To investigate whether women with preeclampsia in multi-fetal pregnancies carry the same long-term CVD risk as women with preeclampsia in singleton pregnancies.

IV. To investigate the pathways behind the excess MI-risk in women with prior PIH, by characterising cardiovascular risk factors in women with a first-time MI in relation to prior PIH status.

Materials and Methods

Overview of the studies

Paper	Study design	Study population	Exposure(s)	Outcome(s)
I	Nested case-control	629 women from the Uppsala University Hospital Biobank of Pregnant Women: preeclampsia (*n*=296), controls (*n*=333)	Plasma cardiovascular biomarkers	Overall preeclampsia. Early- and late-onset preeclampsia.
II	Nested case-control	890 women from the Uppsala University Hospital Biobank of Pregnant Women: preeclampsia (*n*=273), SGA (*n*=106), preeclampsia and SGA (*n*=31), controls (*n*=480)	Plasma cardiovascular biomarkers	Preeclampsia, SGA brith, combined preeclampsia and SGA.
III	Register-based cohort study	892 425 nulliparous women in Sweden	Preeclampsia and multiple pregnancy	Cardiovascular disease
IV	Register-based cohort study	17 814 women from the MBR with prior PIH (*n*=1 753) or prior normotensive pregnancies (*n*=16 061) experiencing their first MI.	PIH	Cardiovascular risk factors

SGA; Small for gestational age, MBR; Medical birth register, PIH; Pregnancy-induced hypertension, MI; Myocardial infarction.

Data Sources

Papers I and II

In Papers I and II, the source population was pregnant women from the population-based Uppsala University Hospital Biobank of Pregnant Women (138). Women aged 18 or older were invited to donate a blood sample for the biobank when attending their early second-trimester routine ultrasound scan at the University Hospital in Uppsala. If they accepted and gave written consent, a venous blood sample was collected in ethylenediaminetetraacetic acid-containing tubes. The samples were centrifuged (1 500g for 10 min) and stored at -70°C within 2 hours after sampling.

About 97% of the pregnant population in Uppsala County participates in the routine ultrasound examination, and all routine ultrasound examinations are performed at Uppsala University Hospital [16]. Previous data show that approximately 50% of the pregnant population in Uppsala County donated blood samples to the biobank during the study period (2007–2018) (138). However, women included in a similar biobank were more often born in Sweden, had an education >12 years and smoked less, in comparison to those who declined participation (153).

Medical records were reviewed for data collection on maternal, pregnancy and infant characteristics, as well as validation of the diagnosis of preeclampsia.

Papers III and IV

In both Papers III and IV, the source populations were derived from the MBR. These studies obtained data from several Swedish registers. In Sweden, researchers may apply for access to multiple national registers held by the Swedish National Board of Health and Welfare and Statistics Sweden. It is mandatory for health care to report to these registers and no consent is needed from individuals. After ethical approval, pseudonymised data can be available for research. In Sweden, each individual is assigned a personal identification number (PIN), consisting of the date of birth and a four-digit number. This PIN enables linkage between different national registers (139).

In Papers III and IV reviewed in this thesis, the Swedish National Board of Health and Welfare provided data from the Medical Birth Register (MBR), the National Patient Register (NPR) and the Cause of Death Register. Statistics Sweden provided access to information from the Total Population Register (TPR), Educational Register, Income Register (Paper III), and National Prescribed Drug Register (Paper IV). For Paper IV, data from a quality register, the Swedish Web-System for Enhancement and Development of Evidence-Based Care in Heart Disease Evaluated According to Recommended Therapies (SWEDEHEART, 1995) was also used.

Swedish Medical Birth Register

The MBR, holds data on demographics, reproductive history, pregnancy, birth, and neonatal period, covering> 98% of all deliveries in Sweden since 1973 (140). Data are prospectively collected starting at the first antenatal visit and throughout pregnancy, birth and the neonatal period. It includes data regarding the mother, such as age, smoking habits, parity, and pregestational diagnoses (recorded by the midwifes at the first antenatal visit through checkboxes). Further, it holds diagnoses for both the mother and infant at discharge from the delivery hospital, e.g., maternal diagnoses, gestational length, birth characteristics, birthweight, gestational length at birth, single/multiple birth and stillbirth. All diagnoses were classified according to the Swedish version of the International Classification of Diseases (ICD) (ICD-8 until 1986, ICD-9 between 1987−1996, and ICD-10 from 1997 and onwards) (140, 141).

Swedish National Patient Register

The National Patient Register holds information on specialised in- and outpatient care, such as dates of hospital admission and discharge diagnoses classified according to ICD codes (since1964, nationwide since 1987) (141). Since 2001, out-patient visits in specialised health care have also been included (142). However, the National Patient Register does not cover conditions diagnosed in primary health care (142).

Swedish National Cause of Death Register

The Swedish Cause of Death Register (since 1952) compromises data on all deaths of Swedish residents (6).

Swedish Total Population Register

The Swedish Total Population Register (since 1968), holds information on the population, e.g., civil status, country of birth and migration. Data on births, deaths, and civil status are reported by professional and administrative personnel and regarded as being of high quality. However, data on migration might be compromised since reporting of this data depends on the individual (143).

Swedish Educational Register

The education register (since 1985) holds longitudinal data on the highest level of education and educational orientation, of the Swedish population.

Swedish Income Register

The income register (since 1968) holds information about earnings and payment data.

Swedish National Prescribed Drug Register

The National Prescribed Drug Register (since 2005) provides information on all prescribed drugs dispensed in Sweden.

SWEDEHEART

The SWEDEHEART registry (since 1995) includes patients admitted to the hospital due to suspect MI. All patients are informed about their participation in the register, and that they have the right to decline, but written consent is not required. Information is collected prospectively for several variables, including: cardiovascular risk factors, medical treatment prior to admission, biochemical markers (routine blood samples) and other clinical features (28). In 2006, the register covered 71 hospitals and had gained a mean coverage of

95% for all hospitalised patients. In 2008, full coverage of all 74 hospitals that provide coronary care units was reached.

In comparison to national registries that are nationwide and population based, quality registries like SWEDEHEART are focused on patients with specific conditions, e.g., acute coronary syndrome, and cover disease specific data. By tracking clinical features, treatments, and outcomes, this quality register aims to improve the quality of the patient care delivered by providing evidence-based treatments. Data collection enables feedback to the care giver, monitoring of adherence to guidelines, and research.

Study populations and study design

Papers I and II

From the Uppsala University Hospital Biobank of Pregnant Women, we included women with singleton pregnancies who had donated a blood sample between 16+0–20+6 weeks gestation and had given birth at 22 weeks' gestation or later. Due to the explorative nature of the study, and to minimise confounding, women with conditions that could alter the examined biomarkers (chronic hypertension, pre-gestational or gestational diabetes, known renal disease, and ongoing treatment with immune- or coagulation-modulating medication (including aspirin) or lithium) were excluded. In Sweden, the use of aspirin for preeclampsia prevention was relatively rare during the study period, approximately 1–2% of nulliparous women receiving this treatment (11), and women with a history of late-onset preeclampsia were usually not offered aspirin prophylaxis.

In Paper I, cases were women who developed preeclampsia later on in the index pregnancy (n=296) (Figure 4).

In Paper II, cases were women who in the index pregnancy developed preeclampsia only (n=273) or gave birth to an SGA infant only (n=106) or both conditions combined (n=31) (Figure 4).

In both papers, controls were healthy pregnant women without a history of preeclampsia who continued a normal pregnancy after blood sampling (study I n=333, study II, n=480). Normal pregnancy was defined as a pregnancy without hypertension, isolated proteinuria, cholestasis of pregnancy, isoimmunisation, maternal thromboembolism, oligo- or polyhydramnios, placental abruption, preterm birth (before 37 weeks' gestation), or birth of an infant born SGA or stillborn (Figure 4). In both papers cases and controls were individually matched on BMI and parity. As predefined quality control, we also assessed group-level balance in maternal and technical characteristics (maternal age, first-trimester smoking status, gestational week at blood sampling, and storage time of samples) to avoid systematic differences between groups. After reviewing medical records for data collection on maternal, pregnancy and infant characteristics, as well as validation of diagnosis of preeclampsia, some samples were excluded.

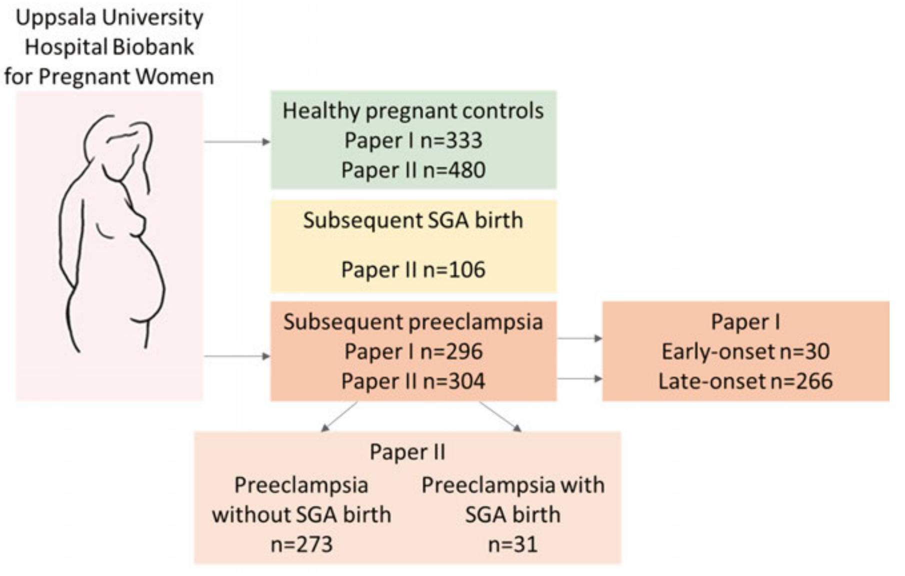

Figure 4. Flow chart of study population in Papers I and II.

Papers III and IV

In study III, we included all women born in Sweden between 1 January, 1950 and 32 December 1971 who had their first singleton birth between 1 January 1973 and 31 December 31 2010. After exclusions, the study population consisted of 892 425 nulliparous women who were living in Sweden with singleton or multi-fetal pregnancies, without an existing diagnosis of CVD (ischemic heart disease, heart failure, or cerebrovascular disease according to ICD diagnoses) at start of follow-up 3 months after birth (Figure 5).

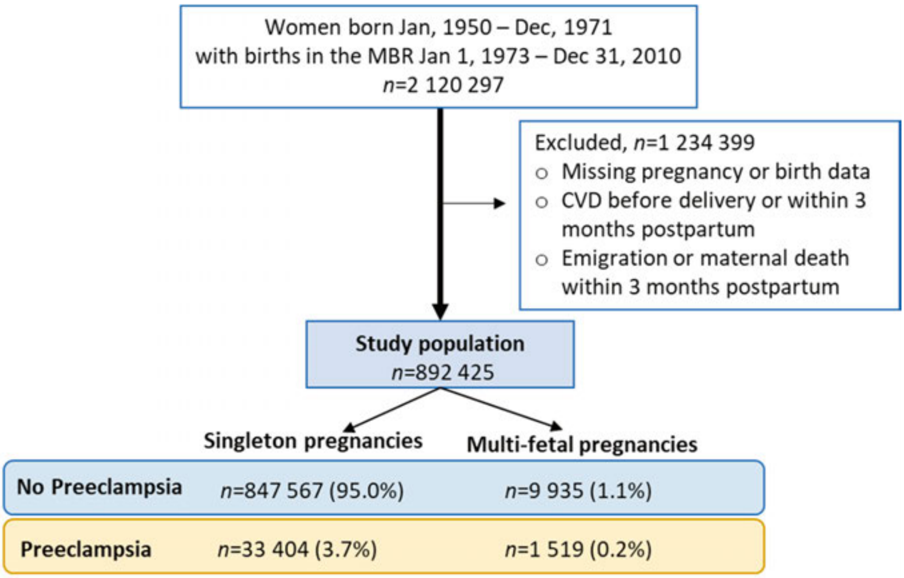

Figure 5. Flow chart of study population in Paper III.

Study IV included all women in Sweden giving birth to a singleton infant at ≥22+0 gestational weeks, from 1 January 1973 until 31 December 2019 (*n*= 2 439 833). Women with an MI before last birth were excluded (*n*= 275). To study cardiovascular risk factors post-pregnancy, we also excluded those with one of the following diagnoses in the MBR (indicating the cardiovascular risk factor already at pregnancy): pregestational hypertension, diabetes mellitus, chronic kidney disease, or systemic lupus erythematosus. Thereafter, 2 399 752 women remained.

The follow-up was conducted through the SWEDEHEART register, and started January 1, 2006 since information on systolic and diastolic blood pressure, plasma (p) glucose, serum (s) lipids, and s-creatinine were not included in the register before that date (29). Inclusion required confirmation of a MI in SWEDEHEART by a corresponding ICD code in either the National Patient Register or the Cause of Death Register within 1 week of admission to a coronary care unit. To ensure inclusion of only first-time MIs, women with a prior MI-diagnosis in the National Patient Register before the first registered MI in SWEDEHEART were excluded. The final cohort consisted of 17 814 women giving birth 1973–2018, without chronic diseases recognised as cardiovascular risk factors during pregnancy and thereafter developing a first-time MI between 2006 and 2020 (Figure 6).

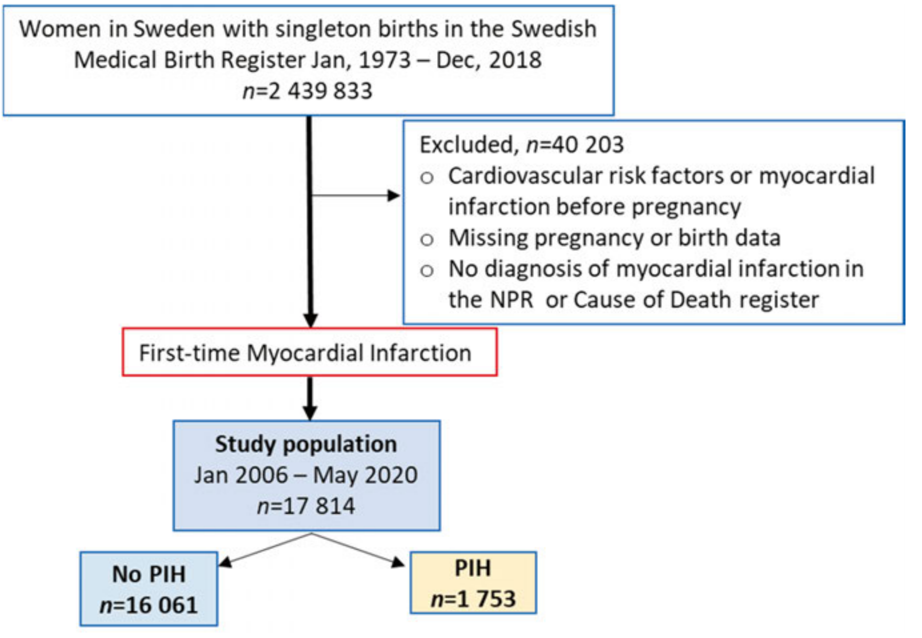

Figure 6. Flow chart of study population Paper IV. PIH indicates pregnancy induced hypertensive disorders; NPR, Swedish National Patient Register.

Exposures

Papers I and II

The exposure in Papers I and II 92 cardiovascular and inflammatory biomarkers assessed by Olink's Proseek multiplex Cardiovascular II (CVD-II) Panel (144). The data were reported as normalised protein expression (NPX) on a Log_2-scale, i.e., relative protein values, where a one unit increase in NPX corresponds to a doubling of the protein concentration (145). The full names of the biomarkers are presented in Papers I and II.

Paper III

The exposure was defined as a maternal diagnosis of preeclampsia in nulliparous women, combined with the plurality of pregnancy (singleton or multifetal). The primary exposure group was multi-fetal pregnancies with preeclampsia. Women were identified by corresponding ICD 8, 9, or 10 codes for preeclampsia registered in the MBR. The birth record provided information on the plurality of pregnancy.

The reference category were nulliparous women with singleton pregnancies without preeclampsia. All combinations of plurality, with or without a diagnosis of preeclampsia, were compared to the reference category.

Paper IV

The key exposure was defined as a diagnosis of prior PIH, identified by corresponding ICD 8, 9, or 10 codes registered in the MBR. During the study period the definition of gestational hypertension remained the same (blood pressure ≥140/90 mmHg) without proteinuria, occurring after 20 weeks' gestation). For preeclampsia, the definition was new-onset hypertension during pregnancy combined with proteinuria or oedema (ICD 8), and only proteinuria (ICD 9 and 10) during the study period.

In sub-analyses, cardiovascular risk factors were further examined in relation to the following separate PIH subtypes identified through MBR data: gestational hypertension or preeclampsia, PIH with preterm or term birth (<37+0 respectively ≥37+0 weeks' gestation), single or recurrent PIH.

For all analyses, the reference category was defined as singleton normotensive pregnancies.

Outcomes

Paper I

The outcome group was defined as women with singleton pregnancies with subsequent preeclampsia, defined as early- or late-onset (<or≥34 weeks' gestation). A diagnosis of preeclampsia was identified by corresponding ICD codes in the medical journal, as reported by the responsible physician at postpartum discharge. For women with repeated pregnancies complicated by preeclampsia that were included in the biobank, the pregnancy with earliest onset of preeclampsia was prioritised. Preeclampsia was defined a new-onset of hypertension (SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg) measured on two subsequent occasions at least 4 hours apart and proteinuria (≥300 mg/24 hour or ≥2+ on a dipstick test) after 20 weeks' gestation (20).

Controls were defined as healthy pregnant women without prior preeclampsia and with subsequent normal pregnancy, i.e., without development of hypertension, preterm birth (<37 weeks' gestation), birth of an infant born SGA, or stillborn.

Paper II

The outcome groups were defined as: (1) preeclampsia (with a non-SGA infant), (2) SGA birth (without preeclampsia), and (3) combined preeclampsia and SGA birth. A diagnosis of preeclampsia was defined as stated above in Paper I. The definition of SGA birth, which was used as a proxy for FGR, was birth weight <−2 standard deviations (SD) for gestational age and sex (corresponding to the 2.3rd percentile) (146).

The definition for controls were the same as that used in Paper I.

Paper III

The outcome was defined as the first CVD event (1973−2010), identified either as a diagnosis in the National Patient Register or as an underlying cause of death in the Cause of Death Register. CVD events included ischaemic heart disease, heart failure, or cerebrovascular disease. To avoid misclassification of outcomes in the National Patient Register, a CVD diagnosis was defined according to the following criteria: 1) one ICD code for CVD in the inpatient register; 2) one ICD code for CVD in the outpatient register followed by an inpatient CVD code within 1 year; or 3) two outpatient codes recorded within 1 year.

Paper IV

The investigated outcomes at first-time MI were the cardiovascular risk factors 1) age, 2) BMI, 3) current smoking, 4) antihypertensive drug treatment 5) systolic blood pressure, 6) diastolic blood pressure, 7) antidiabetic drug treatment, 8) p-glucose, 9) lipid-lowering drug treatment, 10) s-triglycerides, 11) s-high-density lipoprotein (HDL) cholesterol, 12) s-low-density lipoprotein (LDL) cholesterol, and 13) s-creatinine.

BMI was calculated as weight/(height^2) at admission for MI. Former smoking was classified as a woman who stopped smoking at least 1 month before MI. Chronic hypertension in the register is defined as prior or ongoing antihypertensive drug treatment at time of the MI. This diagnosis was also confirmed with at least one dispensed prescription of a antihypertensive drug (ATC codes in Table S4) registered in the National Prescribed Drug Register during the year before the MI. Systolic and diastolic blood pressure levels were recorded at hospital admission for the first-time MI, or at the emergency care centre before admission. In women with no antihypertensive drug treatment, systolic blood pressure ≥ 140mmHg and/or diastolic blood pressure ≥ 90mmHg was used as a proxy for undiagnosed chronic hypertension (93).

Use of antidiabetic or lipid-lowering drug treatment was used as proxies for diabetes mellitus and hyperlipidaemia, diagnosed before the first MI. These cases were identified by at least one dispensed prescription registered in the National Prescribed Drug Register of a drug related to the treatment of these conditions (ATC codes are presented in Table S4) during the year before the MI. Non-fasting blood samples for glucose and creatinine were taken at admission or shortly thereafter. Fasting s-lipids were measured within 24 hours after admission. S-LDL cholesterol was calculated using Friedewald's formula (147). All analyses were performed at hospitals with accredited medical laboratories as part of routine the clinical management of MI.

Laboratory Analyses

Plasma samples in Papers I and II were analysed at the Science for Life Laboratory, Uppsala, Sweden, using Olink's Proseek multiplex Cardiovascular II (CVD-II) Panel. The proximity extension assay (PEA) allows antibodies to pairwise bind to the target protein in the sample. A proximity-dependent DNA polymerisation event produces a new target sequence that is detected and quantified with real-time polymerase chain reaction (PCR). Each assay plate included cases, interplate controls (allowing for adjustments of any differences between runs), and negative controls (144, 148).

The limit of detection (LOD) was defined as background plus 3 standard deviations, estimated from negative controls and calculated separately for each plate. To increase statistical power and preserve distributional properties, we retained all measured NPX values, including those below the LOD, rather than imputing. The exception was proteins with NPX values above the upper quantification limit together with cropped violin plot distributions, indicating a signal drop caused by protein excess relative to the antibody probes (hook-effect). These proteins (pregnancy-associated plasma protein A and interleukin-27) were excluded from the analysis in Paper II to avoid misinterpretation due to falsely lowered values (148).

Statistical methods

Paper I

Background characteristics were compared between cases and controls using Pearson's chi-squared test for categorical variables and the Student *t* test for mean of continuous variables.

To explore the predictive importance of the biomarkers the Boruta algorithm, which is a machine learning approach, was applies. This method is based on Random Survival Forest (RF) model, which provided an unbiased grading of the predictive importance of all variables, including: biomarker NPX levels and clinical characteristics (parity, maternal age, first trimester BMI, smoking habits, SBP and DBP, and gestational age at blood sampling) (29). The Boruta algorithm was then applied to identify variables with importance exceeding that expected from random association with preeclampsia development. It performed repeated RF runs with added shuffled copies of the original variables (shadow variables) and iteratively compared their importance. Variables consistently outperforming the shadow variables are classified as confirmed, those performing worse as rejected, and those in between as tentative. Separate analyses for overall preeclampsia, early- and late-onset preeclampsia were performed.

Unadjusted and adjusted logistic regression analyses estimated the direction of the individual association of each of the 92 biomarkers to preeclampsia, and its subtypes. Bonferroni's post hoc test was applied to correct for multiple testing (92 proteins), and p-values under 0.05/92 were considered significant. Adjustments were made for the following confounders (identified by drawing and analysing a directed acyclic graph): parity, maternal age, first trimester BMI, first trimester smoking habits, first trimester systolic and diastolic blood pressure, and gestational age at blood sampling. Estimates were presented as adjusted odds ratios (aOR) with 95% confidence intervals (CI). The regression analyses were run on complete data sets.

Statistical analyses were performed in R version 4.0.2 and the Statistical Package for the Social Science (SPSS) Statistics 27.0.

Paper II

In Paper II, relative NPX values were reported as median and interquartile ranges (25−75%). To assess overall differences in protein values across the four groups (i.e., controls, subsequent preeclampsia, SGA birth or combined disorders), a false discovery rate (FDR) adjusted Kruskal Wallis test was applied ($p<0.0001$). For proteins with significant group differences, Dunn's Bonferroni post-hoc test was used to compare each outcome group with controls. In addition, we evaluated differential expression between each outcome group and control group by calculating fold changes. We defined top proteins as FDR <0.05 and $\log_2$ fold change > 0.1 or < −0.1.

To confirm that differences in baseline characteristics did not drive our results, we conducted unadjusted and adjusted multinominal logistic regression analyses on proteins that differed between groups. We adjusted for confounders identified though a directed acyclic graph, including parity, maternal age, first trimester BMI, smoking habits, systolic and diastolic blood pressure, and gestational age at blood sampling. The logistic regression analyses were run on complete data sets.

Statistical analyses were done in Statistical Package for the Social Science (SPSS®) Statistics version 25.

Paper III

Data were analysed using time-to-event methods for women giving birth from 1973 to 2010. We adjusted for key covariates that may be associated with both preeclampsia in first pregnancy and plurality, as well as long-term cardiovascular disease risk. Some important confounders could be accounted for throughout the entire study period, while others were only available for the later part of it. Therefore, time-restricted analyses were conducted.

The first CVD event during follow-up was studied. Time at risk was accumulated from entry into the cohort until CVD event or censoring. Survival analysis was conducted using attained age as the underlying time scale, reflecting its direct relevance to CVD risk.

The exposure was analysed as a categorical variable, and its associations with CVD incidence were assessed using the Cox proportional hazard models, yielding hazard ratios (HR) with 95% CIs. The models were analysed unadjusted and adjusted for confounders (available since 1973) such as maternal age at birth, chronic hypertension before first birth, level of education, and time period of birth.

Earlier studies suggest a J-shaped association between parity and later CVD risk. Since subsequent pregnancies may mediate this association, and women with multifetal pregnancies often have fewer total pregnancies, we did not adjust for parity beyond the first pregnancy, as it occurs after exposure, and is not a confounder. Adjusting for it could underestimating the true effect. Similarly, preterm birth was defined as a confounder.

Statistical analyses for Paper III were performed using the SAS software version 9.4. and STATA software version 14.

Paper IV

Descriptive maternal baseline characteristics were compiled and presented as absolute numbers, medians and interquartile range (IQR) or as frequencies (%). Missing data was handled as missing in all analyses. Analyses of blood pressure, p-glucose and s-lipid levels were conducted separately for women with and without antihypertensive, antidiabetic and lipid-lowering drug treatment, respectively. For comparisons between cardiovascular risk factors between women with prior PIH and normotensive pregnancies, the Wilcoxon rank sum test was used for non-normally distributed data, and Pearson's chi-square test was used for categorical data.

To compare cardiovascular risk factors in women with first-time MI in women with prior PIH and those with prior normotensive pregnancies, we fitted a binary logistic regression model. PIH status (yes/no) was the dependent variable, with cardiovascular risk factors serving as the independent variables. By including all risk factors simultaneously in the model, we accounted for their mutual influence, i.e., allowing for a comparison of each factor's association with PIH status while adjusting for the other factors. Coefficients were interpreted as indicators of relative prevalence: a positive coefficient (i.e., adjusted odds ratio [aOR]>1) suggests that the variable is a more common, whereas a negative coefficient (aOR<1) indicates that the variable is a less common risk factor among women with prior PIH, than among those with prior normotensive pregnancies. To facilitate comparisons across risk factors, they were standardised prior to analysis, allowing the magnitude of the coefficients to reflect the relative strength of association on a comparable scale (149). The analyses were run on complete data sets.

In sub-analyses, PIH were separated into gestational hypertension and preeclampsia; PIH with and without preterm (<37+0 gestational weeks) birth; and women with single episode and recurrent PIH. The number of women in the unexposed category, i.e., women with normotensive pregnancies, remained constant. The above analyses were repeated for each subtype.

All the analyses were performed using R version 4.4.2 and SPSS® version 25.

Ethical considerations

The studies (Papers I-IV) included in this thesis were performed in accordance with the ethical standards of the Helsinki declaration (150).

Ethical approvals for Papers I and II were obtained from the Uppsala Ethical Review Board, Sweden (Reference numbers: 2007/181, 2010/129 and 2018/251). Informed consent was obtained from all women before inclusion in the Uppsala University Hospital Biobank of Pregnant Women. The women underwent blood sampling when enrolled in the Uppsala University Hospital Biobank of Pregnant Women. The mother and foetus underwent no other physical harm. Each participant received a specific code number for the blood sample. Data is stored in a secure online database. To ensure further security, the code key is stored separately from the data and blood samples.

Paper III was approved from the Regional Ethical Review Board in Stockholm, Sweden (reference numbers: IRB/KI 2012/1325-31/3). Paper IV received approval from the Swedish Ethical Review Authority (Reference number: 2019-04925 approved 28/01/2020, with supplement 2020-04890 approved 28/09/2020). Registry-based studies aggregate extensive data from multiple national registries, necessitating careful consideration of individual privacy and data protection. However, the data were pseudonymised by the National Board of Health and Welfare using a unique serial number upon birth. The researchers had no access to any information that could lead to the identification of any individual. Thus, the possibility of a breach of integrity or privacy is low. Consent requirements do not apply to register studies using pseudonymised data.

Results

Paper I

Among women with singleton pregnancies, those who developed preeclampsia had higher first-trimester systolic and diastolic blood pressures, shorter pregnancy duration, and more commonly gave birth to SGA infants than woman with normotensive pregnancies. In those with subsequent preeclampsia, N=30 (10%) had early-onset preeclampsia.

The Boruta algorithm identified nine variables with stronger than random associations with overall preeclampsia risk, including MMP-12, natriuretic peptide B (BNP), PlGF, first-trimester systolic blood pressure, alpha-L-iduronidase (IDUA), sortilin, growth hormone (GH), kidney injury molecule 1 (KIM1), and mitochondrial carbonic anhydrase 5A (Figure 7).

Seven biomarkers for early-onset preeclampsia and six biomarkers for late-onset preeclampsia were confirmed to have a larger than random association with overall risk of developing preeclampsia (Figure 8A-B).

Adjusted logistic regression models found that decreased levels of MMP-12, BNP, and PlGF, respectively increased levels of IDUA were associated with increased risk of subsequent preeclampsia.

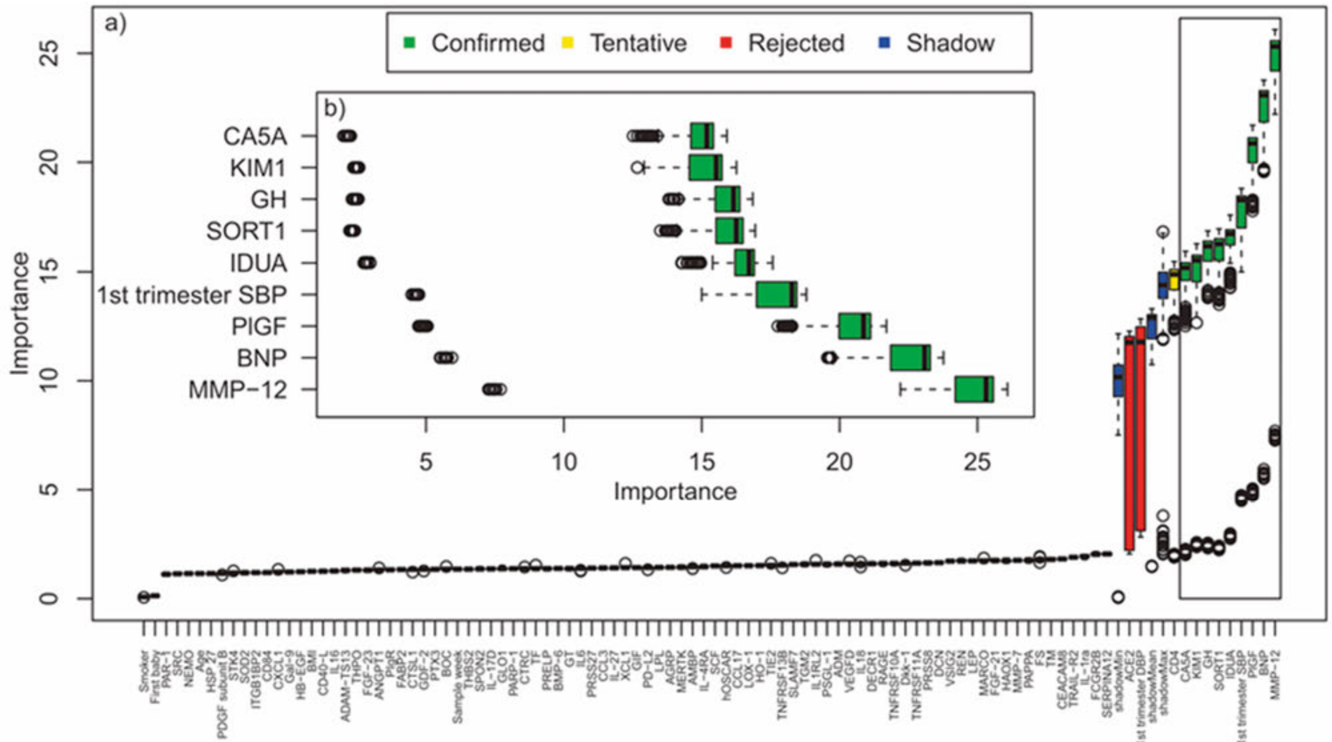

Figure 7. Variable importance for subsequent preeclampsia (*n*=333) by machine learning. Matched on all maternal characteristics except first-trimester blood pressure. The x-axis shows variable importance; the y-axis lists confirmed biomarkers and maternal characteristics. Boxplot centre-lines indicate medians; boxes show the 25th–75th percentiles; whiskers mark the 5th–95th percentiles; white dots are outliers. Green boxes highlight variables outperforming reference levels.

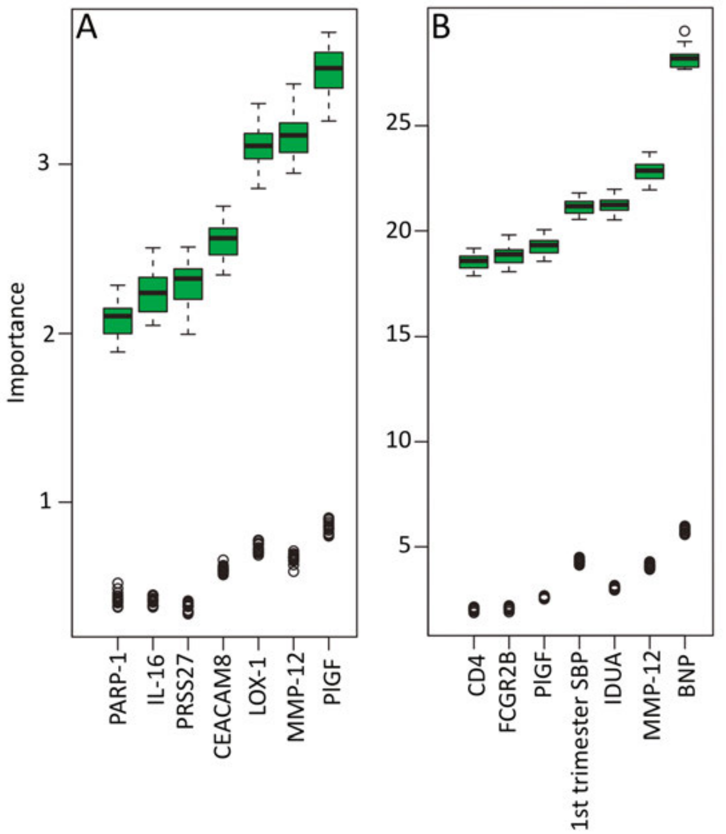

Figure 8. Variable importance for subsequent early-onset (A, *n*=30) and late-onset (B, *n*=296) preeclampsia by machine learning. Matched on all maternal characteristics except first-trimester blood pressure. The y-axis shows variable importance; the x-axis lists confirmed biomarkers and maternal characteristics. Boxplot centre-lines indicate medians; boxes show the 25th–75th percentiles; whiskers mark the 5th–95th percentiles; white dots are outliers. Green boxes highlight variables outperforming reference levels.

Paper II

A total of 890 pregnancies were included in this study. Cases and controls were largely comparable regarding maternal and pregnancy characteristics, with the following exceptions: women with subsequent SGA had lower BMI; gestational age was shorter in women with subsequent preeclampsia or combined disorders; and blood pressure at the first antenatal visit was higher in women who later developed preeclampsia than it was in the other outcomes.

We identified 16 proteins that were differently expressed in women with subsequent preeclampsia than they were, in the other outcome groups, when compared with controls. Among these, 6 proteins were identified as top proteins (absolute $\log_2$ fold change>0.1, Figure 9).

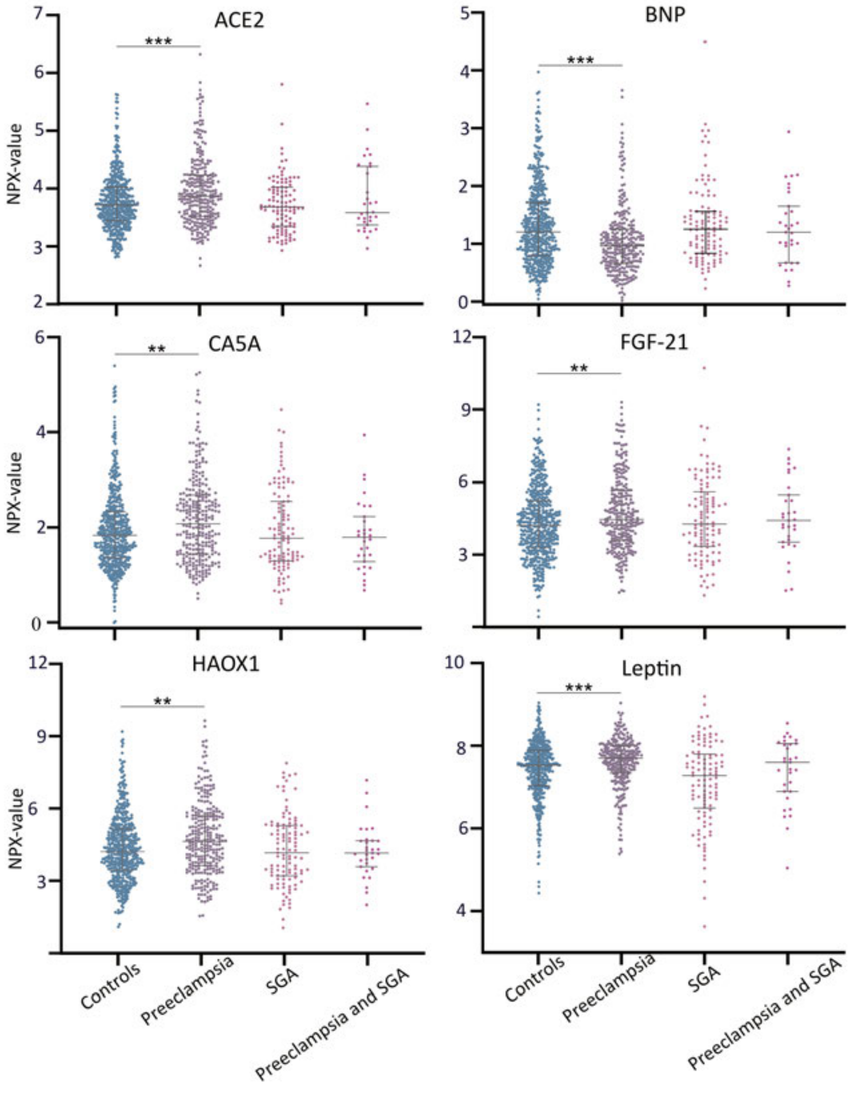

Figure 9. Scatterplots showing top proteins (FDR < 0.05; $\log_2$ fold change > 0.1) differently expressed in women with subsequent preeclampsia compared with controls. Panels show: ACE2 (angiotensin-converting enzyme 2), BNP (B-type natriuretic peptide), CA5A (Carbonic anhydrase 5A, mitochondrial), FGF-21 (Fibroblast growth factor 21), HAOX1 (Hydroxyacid oxidase 1), Leptin. Data are normalised protein expression (NPX, $\log_2$). Horizontal bars indicate median ± IQR. $^{**}p < 0.01$; $^{***}p < 0.001$. SGA indicates birth of small-for-gestational-age infant, defined as birth weight <−2 standard deviations for gestational age and sex.

Two proteins were differently expressed in women with subsequent SGA birth compared to controls. Adenomedullin (ADM), showing lower levels, was identified as a top protein (absolute $\log_2$ fold change<–0.1), Figure 10a.

Interleukin-16 (IL-16) and proteinase-activated receptor 1 (PAR-1) were differently expressed with higher values when comparing women with subsequent combined preeclampsia and SGA birth to controls. Both were identified as top proteins (absolute $\log_2$ fold change>0.1), Figure 10b-c.

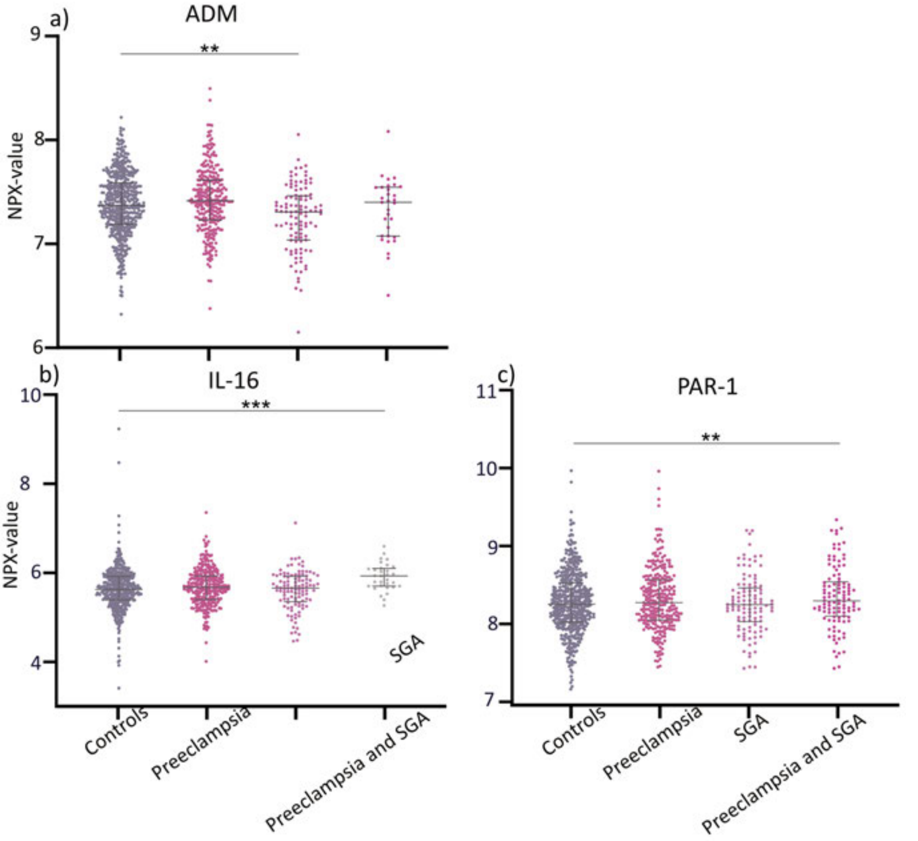

Figure 10. Scatterplots of top proteins (FDR < 0.05; $\log_2$ fold change > 0.1) differently expressed in women with subsequent SGA birth (panel a) or combined PE and SGA birth (Panel b and c), compared with controls. Panels a–c show: ADM (adrenomedullin), IL (interleukin-16)-16, PAR (proteinase-activated receptor 1), MMP (matrix metalloproteinase)-12, and PlGF (placental growth factor), respectively. Data are normalised protein expression (NPX, $\log_2$). Horizontal bars indicate median ± IQR. **p < 0.01; ***p < 0.001. SGA indicates birth of small-for-gestational-age infant, defined as birth weight <−2 standard deviations for gestational age and sex.

When comparing each of the outcome groups to controls, the proteins MMP-12 and PIGF, were differently expressed. Compared to controls, both proteins showed lower NPX values in preeclampsia, SGA birth and combined preeclampsia and SGA birth. The lowest values were found in the last group. MMP-12 and PlGF were both identified as top proteins (absolute $\log_2$ fold change <–0.1, Figure 11) in all comparisons.

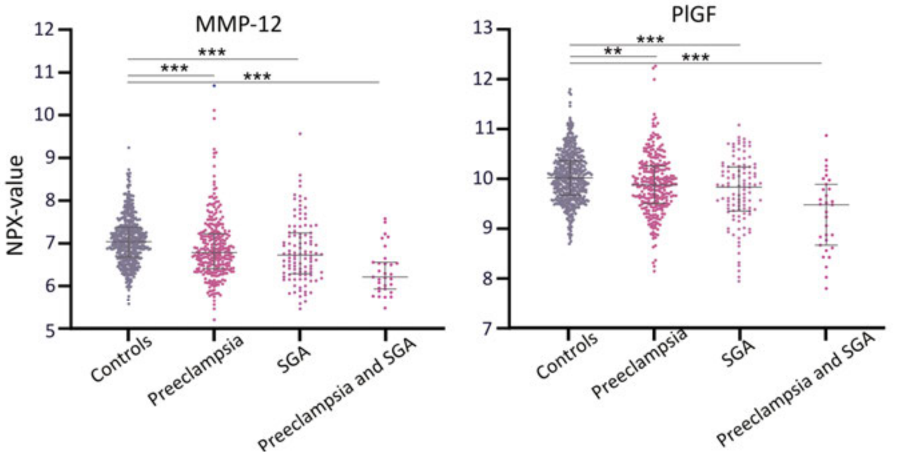

Figure 11. Scatterplots of top proteins (FDR < 0.05; $\log_2$ fold change > 0.1) differently ex-pressed in women with subsequent preeclampsia, SGA birth, or combined PE and SGA birth, compared with controls. Panel a) and b) show MMP (matrix metallopro-teinase-12) and PlGF (placental growth factor) Data are normalised protein expression (NPX, $\log_2$). Horizontal bars indicate median ± IQR. **p < 0.01; ***p < 0.001; SGA indicates birth of small-for-gestational-age infant, defined as birth weight <−2 stand-ard deviations for gestational age and sex.

Paper III

Women with multi-fetal pregnancies compared to those with singleton pregnancies were older and had a shorter mean gestational length. When comparing women with and without preeclampsia, the pregnancy characteristics varied by plurality in the first pregnancy. Among women with singleton pregnancies, those with preeclampsia had a shorter gestational duration, lower maternal education levels, more often pregestational diabetes, and longer durations of infertility compared to those without preeclampsia. Conversely, in women with multi-fetal pregnancies, those with preeclampsia had a longer gestational duration, higher maternal education levels, and less frequently pregestational diabetes than their counterparts without preeclampsia.

Shared risk factors for preeclampsia in both singleton and multi-fetal pregnancies were higher maternal age, higher body mass index, and presence of gestational diabetes mellitus. Smoking was a shared protective risk factor.

The follow-up time ranged up to 38 years. The overall median age at CVD event was 48.4 years. During follow-up, 18 415 women (2.1%) were diagnosed with CVD. The proportion of CVD in the reference category and outcome groups is presented in Table 1.

Table 5. Maternal and pregnancy characteristics according to later compound cardiovascular disease among 892 425 first births 1973-2010

Maternal and pregnancy characteristics of first birth 1973−2010	**Total**	**Compound cardio-vascular disease**	
		N	%
Total	892 425	18 415	2.1
Singleton without preeclampsia	847 567	17 217	2.0
Singleton with preeclampsia	33 404	1 029	3.1
Multi-fetal without preeclampsia	9 935	145	1.5
Multi-fetal with preeclampsia	1 519	24	1.6

In the time to event analysis, after adjusting for maternal age at birth, chronic hypertension before first birth, level of education and time period of birth. The adjusted HR (aHR) of CVD among women with multi-fetal pregnancy and preeclampsia was not statistically different from the hazard among women without preeclampsia in a singleton pregnancy (aHR 1.25 [95% CI, 0.83–1.86]); however, the point estimate was further from the null value of 1, and 95% CIs were wide (Table 6).

Table 6. Adjusted and unadjusted hazard ratios for later compound cardiovascular disease occurring 3 months after birth among 892 425 women with singleton first deliveries without and with preeclampsia and multi-fetal first deliveries without and with preeclampsia 1973−2010

Exposure	**Unadjusted Hazard Ratio (95% CI)**	**Adjusted* Hazard Ratio (95% CI)**
Singleton without preeclampsia	Ref	Ref
Singleton with preeclampsia	1.72 (1.61−1.83)	1.75 (1.64−1.86)
Multi-fetal without preeclampsia	0.84 (0.71−0.99)	0.94 (0.79−1.10)
Multi-fetal with preeclampsia	1.09 (0.73−1.62)	1.25 (0.83−1.86)

*Adjusted for maternal age at birth, chronic hypertension before first birth, level of education, and time-period of birth. Number of observations read: 889 400.

Paper IV

At index pregnancy women with prior PIH had a higher BMI, smoked less and more often gave birth to an SGA infant. The median follow-up time was 33 years. At the time of their first MI, women with prior PIH were younger, had a higher BMI, were more obese, and had lower rates of smoking, compared to those without prior PIH (Table 7).

Women with a first-time MI and prior PIH more often used antihypertensive, antidiabetic or lipid-lowering treatment compared to women with normotensive pregnancies. Further, women with prior PIH had a higher proportion of undiagnosed chronic hypertension compared to those with normotensive pregnancies (Table 7).

In women with a first-time MI and prior PIH, both systolic and diastolic blood pressure were higher in women without antihypertensive treatment compared to women with prior normotensive pregnancies. Among corresponding women with antihypertensive treatment and prior PIH, only diastolic blood pressure was higher than women without prior PIH (Table 7).

Table 7. Cardiovascular risk factors among women with first-time myocardial infarction (MI), separated by prior exposure to pregnancy-induced hypertensive disorders (PIH) or not.

Cardiovascular risk factors	**Prior PIH in any pregnancy**		*p*-value
	No (*n*=16 061)	**Yes** (*n*=1 753)	
Age, years	62.9 (56.3, 68.7)	60.5 (53.3, 67.1)	<0.001
Body mass index, kg/m^2	26.5 (23.5, 30.4)	28.4 (24.7, 32.9)	<0.001
≥30	3 950 (26.9)	639 (40.3)	<0.001
Missing	1 394 (8.7)	166 (9.5)	
Current smoker	5 873 (38)	487 (29)	<0.001
Missing	530 (0.3)	72 (4.1)	
Blood pressure (BP)			
Antihypertensive treatment, yes*	6 554 (40.8)	1 039 (59.3)	<0.001
Systolic BP, mmHg	152 (133, 173)	154 (135, 175)	0.08
Missing	97 (0.6)	16 (0.9)	
Diastolic BP, mmHg	86 (75, 98)	88 (76, 100)	0.006
Missing	294 (1.8)	56 (3.2)	
Antihypertensive treatment, no	9 457 (58.5)	710 (37.4)	<0.001
Systolic BP, mmHg	145 (128, 165)	153 (135, 174)	<0.001
Missing	203 (1.3)	16 (0.9)	
Diastolic BP, mmHg	85 (75, 96)	89 (79, 100)	<0.001
Missing	494 (3.1)	42 (2.4)	
Undiagnosed hypertension¶	6 049 (66.5)	516 (75.5)	<0.001
Missing	360	27	
Plasma glucose			
Antidiabetic treatment, yes†	1 857 (11.6)	314 (17.9)	<0.001
Glucose	10.9 (8.0, 14.6)	10.7 (8.4, 14.9)	0.35
Missing	250 (1.6)	49 (2.8)	
Anti-diabetic drugs, no	14 204 (88.4)	1439 (82.1)	<0.001
Glucose, mmol/L	6.5 (5.7, 7.8)	6.7 (5.8, 8.0)	0.001
Missing	2 092 (13.0)	198 (11.3)	
Serum lipids			
Lipid-lowering treatment, yes#	3 143 (19.6)	449 (25.6)	<0.001
Triglycerides, mmol/l	1.5 (1.1, 2.1)	1.6 (1.1, 2.3)	0.021
Missing	896 (5.6)	135 (7.7)	
HDL cholesterol, mol/l	1.3 (1.0,1.5)	1.2 (1.0, 1.5)	0.014
Missing	747 (4.7)	117 (6.7)	
Lipid-lowering treatment, no	12 918 (80.4)	1 304 (74.4)	<0.001
Triglycerides, mmol/l	1.3 (1.0, 1.9)	1.4 (1.0, 2.0)	0.003
Missing	2 893 (18.0)	291 (16.6)	
HDL cholesterol, mol/l	1.3 (1.1, 1.6)	1.3 (1.1, 1.6)	0.002
Missing	2197 (13.7)	227 (12.9)	

* Indicates pre-known chronic hypertension at admission according to SWEDEHEART, confirmed with at least one dispensed prescription of a blood pressure-lowering treatment during the year before the myocardial infarction; †pre-known diabetes, #pre-known dyslipidaemia, defined as at least one dispensed prescription of antidiabetic respectively lipid-lowering treatment during the year before the myocardial infarction; ¶systolic and/or diastolic blood pressure ≥140/90 at first-time myocardial infarction; HDL, high-density lipoprotein. The Wilcoxon rank sum test was used for comparison of continuous data, and Pearson's chi-square test was used for categorical data.

Binary logistic regression assessed the independent association of each cardiovascular risk factor with PIH status, adjusting for mutual influence of the other risk factors (Figure 12). Among women with PIH the odds of antihypertensive treatment, elevated systolic blood pressure, and elevated BMI were increased compared to women with prior normotensive pregnancies. They had lower odds of high age and smoking.

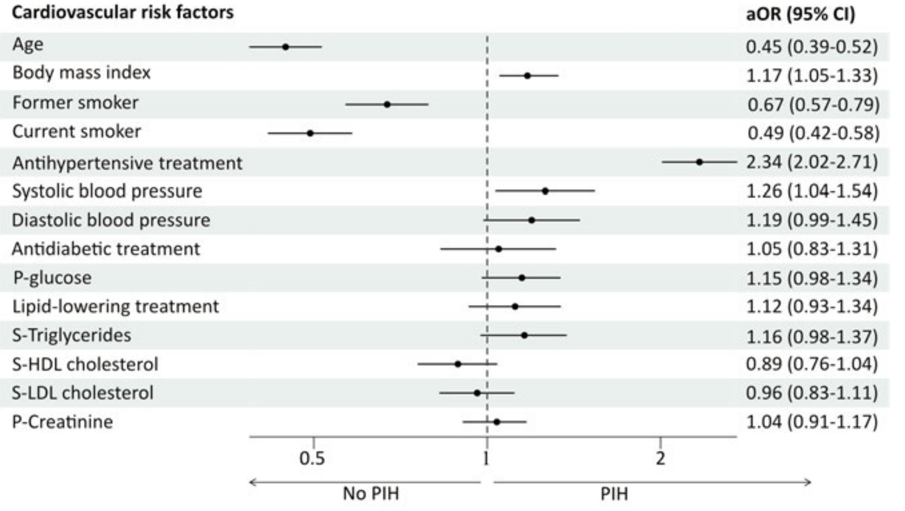

Figure 12. Forest plot presenting the independent association of each cardiovascular risk factor in women with a first-time MI with adjusted odds ratios (aORs) with the 95% confidence intervals (CIs). aOR >1: risk factor more prevalent in women with prior pregnancy-induced hypertensive disorders PIH; aOR <1: less common.

The same analysis was applied to assess cardiovascular risk factors in relation to prior PIH, stratified by a single disorder or recurrent disorders (Figure 13). At the time of first MI, the odds of using antihypertensive treatment were higher in women with a single episode of prior PIH and particularly in those with recurrent PIH compared to normotensive pregnancies. The odds of elevated systolic blood pressure were also increased, whereas the odds of higher age and smoking were lower in both groups.

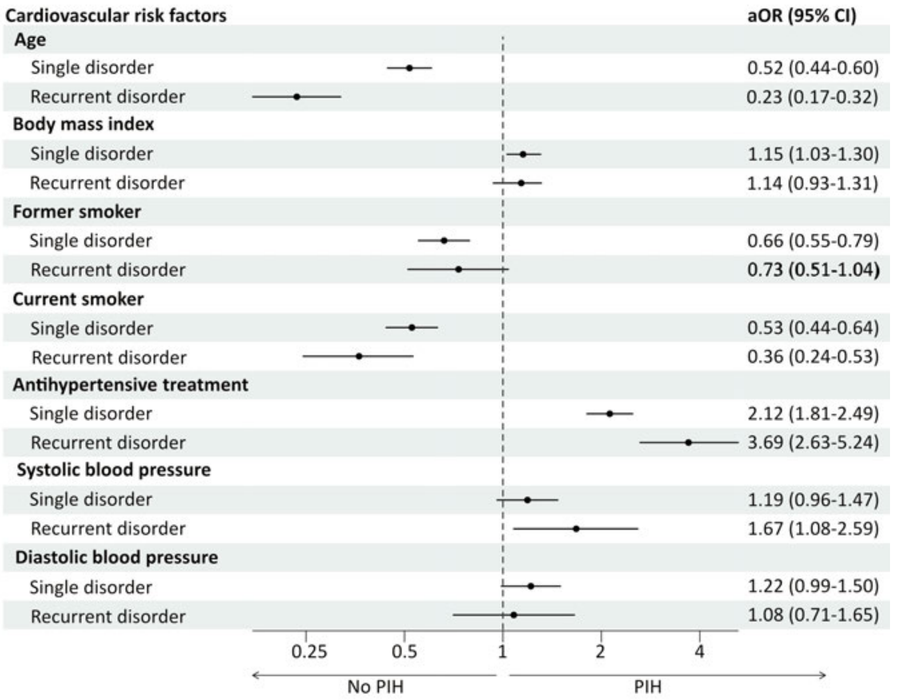

Figure 13. Forest plot presenting the independent association of each cardiovascular risk factor in women with a first-time MI with adjusted odds ratios (aORs) with the 95% confidence intervals (CIs). aOR >1: risk factor more prevalent in women with prior pregnancy induced hypertensive disorders PIH; aOR <1: less common.

These patterns remained similar in the other subtypes (preeclampsia, gestational hypertension, preterm and term preeclampsia) as in the main analyses. The odds of using antihypertensive treatment remained highest in women with prior recurrent PIH compared to normotensive pregnancies.

Discussion

Main findings

This thesis investigated the relationship between preeclampsia and the cardiovascular system. The work focused on identifying biomarkers for preeclampsia prediction and pathophysiology, as well as assessing long-term cardiovascular outcomes following the disorder.

The first Paper of this thesis found that in mid-pregnancy plasma samples MMP-12 showed promise as a predictive biomarker for preeclampsia. With regard to late-onset preeclampsia specifically, BNP and IDUA were also identified as potential predictors.

The second Paper demonstrated that several cardiovascular proteins were dysregulated only in women with subsequent preeclampsia but not SGA birth, compared to women with normotensive pregnancies. In all outcome groups, including combined preeclampsia and SGA birth, we found similar associations to MMP-12 and PlGF, both of which have vascular and placental functions.

The third Paper found that women with a history of preeclampsia in multifetal pregnancies, the long-term CVD risk does not appear to be similarly elevated as in corresponding singleton pregnancies, compared to women with normotensive singleton pregnancies.

Lastly, the fourth Paper showed that in women with a first-time MI, cardiovascular risk factors such as chronic hypertension and elevated BMI were more prevalent among women with prior singleton pregnancy with PIH than in women with normotensive pregnancies. In addition, smoking was less prevalent in these women.

Methodological Considerations

Paper I and II

The study participants in Paper I and II were selected from a cohort of pregnant women enrolled in the Uppsala University Hospital Biobank of Pregnant Women. The main strength of these studies lies in the population-based nature of the Biobank and its' large sample size, which enable a nested case-control study with a substantial number of cases.

Another strength was the prospectively collected data on maternal, pregnancy, and infant characteristics, minimising the risk of recall bias. Furthermore, access to plasma samples from mid-pregnancy, medical records and the relatively large sample sizes, enabled separate exploration of biomarkers prior to clinical symptoms of preeclampsia (late- and early-onset), SGA birth, and combined disorders. Another strength was that all diagnoses were confirmed by review of medical records.

The study design may have introduced selection bias. During the study period, approximately 40% of the pregnant population in Uppsala County donated blood samples to the Biobank, with inclusion depending on nurse availability and participant willingness. Prior research shows that Biobank participants were more often born in Sweden, had >12 years of education, and smoked less than non-participants (151). Because only healthy women were enrolled and preeclampsia diagnoses were strictly validated, fewer cases were reported compared to national registers. Although the number of the combined outcome preeclampsia with SGA birth exceeded previous studies, it was relatively low, which may have affected power. The stricter definition of SGA, −2SD as opposed to the 10^{th} percentile, was chosen to enhance specificity in identifying infants with FGR. Given the outcome group sizes, differences in altered protein levels may reflect limited statistical power due to small sample size.

Both studies have some limitations regarding generalisability. First, the partly matched design (BMI and parity) between cases and controls was selected to minimise factors that could confound the origin of the cardiovascular biomarkers (152). However, matching itself does not control for confounding by the matching variables and can even introduce new confounders. Therefore, we adjusted for BMI and parity in the analyses (153). Second, the study's relatively homogeneous ethnic composition limits generalizability to other populations. Third, eligibility criteria excluded women with conditions affecting cardiovascular and inflammatory proteins (e.g., chronic hypertension, pregestational diabetes, and gestational diabetes), and comparisons were restricted to women with healthy pregnancy outcomes. The weakness of this design is an overestimation of the results, as protein dysregulation could be more pronounced in women with pre-pregnancy conditions. Moreover, results could be different if the reference group included those who later developed complications like gestational hypertension. However, given the explorative nature of both studies, a homogeneous population was preferred to reduce confounding factors (152).

One limitation of the Boruta algorithm used in Paper I is that it does not provide information about the direction or magnitude of each variable's effect. This was addressed with logistic regression analysis, which allows for interpretation of both the strength and direction of associations.

Papers III and IV

Papers III and IV were nationwide register-based cohort studies. Women were followed from birth in the index pregnancy until a CVD event, death, migration or end of follow-up. The population-based design allowed analysis of rare exposures and detailed multiple outcomes, and the larger sample size provided greater power than have many previous studies.

By refining the study cohorts to exclude individuals with prior diagnoses of CVD and due to a long follow-up period, the study design enabled investigation of CVD outcomes in women who were initially at low cardiovascular risk during pregnancy.

As in all observational studies causal inference cannot be established due to the lack of randomisation. Findings should therefore be considered as associations. To account for confounding, variables were selected based on a directed acyclic graph (DAG) (Paper III), *a priori* knowledge and register availability (154). However, some relevant confounders were not available across the entire study period (Paper III), but restricted analysis yielded similar results.

A major strength of using registers, was the merged high-quality population-based national and quality register data, enabling comprehensive analysis of CVD and cardiovascular risk factors in relation to plurality of pregnancy and PIH disorders. In the national health registers, all individuals are automatically included. Quality registers typically use an opt-out process i.e., individuals are automatically included, but can choose to decline participation, although few actually do. This enabled inclusion of all women who developed CVD (Paper III) and MI (Paper IV). In addition, the registers have high coverage and, for several diagnoses, good validity. For example, variables in the MBR have shown good validity for ICD-9 and ICD-10 (used in both papers), with high positive predictive values for gestational hypertension and preeclampsia (84% and 93%) (155-157). The data used from the SWEDEHEART register in Paper IV have also shown high internal validity (158).

Furthermore, both papers were exposed to the possibility of misclassification bias. This was addressed by increasing the accuracy of the included CVD diagnosis by using both in- and out-patient registers (Paper III), and a MI diagnosis in both SWEDEHEART and the National Patient Register or the Cause of Death Register (Paper IV). Also, a diagnosis of chronic hypertension could not be assessed directly in Paper III (as outcome) or Paper IV (as mediator), due to lack of primary care data. Therefore, antihypertensive drug treatment (the Swedish National Prescribed Drug Register) was used as a proxy, together with a diagnosis of hypertension in SWEDEHEART to reduce the risk of misclassification. However, in Paper III, we did not have access to the Swedish National Prescribed Drug Register. Further, using a blood pressure from an emergency setting as a proxy for chronic hypertension (Paper IV) may overestimated the actual diagnosis of chronic hypertension. Nevertheless, screening for hypertension in emergency hospital environment consistently identifies groups of patients with previously undiagnosed hypertension (159).

A specific limitation of Paper IV is that follow-up began in 2006 due to the limited availability of data on cardiovascular risk factors prior to that year. As a result, some women who experienced MI before 2006 may have been missed, potentially leading to an underestimation of risk and introducing selection and survival bias. However, since cohort entry occurred at different time points, some individuals entered the study closer to the start of follow-up. This may have mitigated, though not entirely eliminated, the risk of such biases.

Experimental considerations

Proximity extension assay

The Olink Proseek multiplex CVD-II Panel utilizes proximity extension assay (PEA) technology, enabling high-throughput, multiplex protein analysis with high specificity and sensitivity. A major strength was the simultaneous measurement of 92 cardiovascular and inflammatory proteins, requiring a low sample volume (1 µl of plasma) due to signal amplification. Furthermore the PEA technology allows measurement of protein values on a wider dynamic range than other technologies (160).

A limitation of this panel is that its quantification limits are calibrated using samples from a general non-pregnant population, including both healthy and diseased individuals. Protein levels in healthy pregnant-women may differ from a non-pregnant population due to physiological changes (161). This can affect the accuracy and interpretability of the measurements, especially if the dynamic range or the quantification limits of the panel are not optimised for very low or high abundance of protein levels during pregnancy.

The Olink Proseek multiplex CVD-II Panel generates relative rather than absolute protein values, which is a limitation. However, a previous study using a similar Olink multiplex CVD assay demonstrated excellent correlation between relative and absolute values of PlGF (74).

Research in context and implications

Mid-pregnancy cardiovascular biomarkers

Biomarkers for preeclampsia prediction

Improving prediction models in early pregnancy for preeclampsia, particularly late-onset, requires identification of potential biomarkers that may be incorporated in multivariable prediction models (70, 162). By analysing the individual predictive importance of 92 cardiovascular and inflammatory biomarkers for preeclampsia, we found several biomarkers that may be valuable in future prediction models.

Out of these biomarkers, only PlGF have previously been studied in first- and second trimester prediction models, with promising results for predicting preterm preeclampsia (163, 164). This aligns with our results on PlGF as a predictive biomarker for early-onset preeclampsia. Although prediction of late-onset preeclampsia remains modest in current prediction models with PlGF, we confirmed PlGF as a potential predictive biomarker for late-onset preeclampsia. However, it may require combination with other biomarkers to improve specificity in prediction models. The separate value of PlGF in prediction of late- and early-onset preeclampsia, suggest that despite sharing clinical features, their associations with pre-existing angiogenic imbalance are distinct (48).

In addition, MMP-12 emerged as a novel predictive biomarker for preeclampsia and both subtypes. MMPs are engaged in endometrial remodelling and invasiveness of trophoblast cells, and are released from inflammatory and vascular smooth muscle cells (165-168). Different members of the MMP family have been investigated in pregnancy and preeclampsia, although to a limited extent (74, 169). However, there is only one study that found similar associations between preeclampsia and decreased levels of MMP-12. In addition, they found that MMP-12 levels increased from the first to the second trimester (170). This aligns with our results on decreased MMP-12 levels associated with both early- and late onset preeclampsia. However, given the findings in the previous study, it is possible that the association between decreased MMP-12 levels and preeclampsia would have been even stronger if first-trimester samples had been analysed. Currently, there are no other studies on MMP-12 and preeclampsia prediction. However, a smaller study (n=79 preeclampsia cases) identified MMP-7 as a first- and second-trimester biomarker for late-onset preeclampsia, which contrast the findings of our study where it was not identified as predictive (169). The different results could partly be due to our strictly selected population without pre-pregnancy diseases.

BNP emerged as a specific predictive biomarker only for late-onset preeclampsia. This protein is a well-recognised biomarker for cardiac failure (171). The results on that decreased BNP levels associated to overall as well as late-onset preeclampsia, aligns with previous studies on N-terminal (NT)-

proBNP in the first and second trimesters, including one conducted by our research group using partly the same cohort (171-173). Comparable to our results, one study on first-trimester levels on NT-proBNP, which is cleaved together with BNP from their precursor proBNP, found no association to preterm preeclampsia (174). This strengthen our results on that BNP is not a suitable predictor for early-onset preeclampsia. BNP has primarily been evaluated as a third trimester biomarker for late-onset preeclampsia, showing limited clinical value (74, 175). Given that late-onset preeclampsia composes the majority of preeclampsia cases, the discriminative potential of BNP regarding women at risk for late-onset preeclampsia need further investigation in early prediction models.

Ultimately, achieving first-trimester identification is essential to enable preventative strategies for preeclampsia. We evaluated the predictive performance of biomarkers in the early second trimester. While prediction before 16 weeks of gestation is considered optimal for preventative aspirin treatment, especially for early-onset disease, second-trimester models are still valuable for identifying women who could benefit from individualized follow-up.

The machine-learning model assessed, was limited to relative biomarker values, pre-decided biomarkers and a few maternal characteristics. This necessitates further evaluation of identified biomarkers generalizability and performance using absolute values. Additionally, their clinical applicability should be assessed within multimodal prediction models, along with both biophysical and biochemical biomarkers. If an acceptable performance is reached in such model, the biomarkers may be considered clinically reliable after validation in different populations.

Protein expression before preeclampsia and SGA birth

The origins of preeclampsia and SGA birth overlap to some extent and both conditions are associated with cardiovascular risk factors, adverse cardiovascular health, as well as mal-placentation (5, 23, 48, 109, 119). However, it remains unclear why some individuals develop one condition, the other, or both. When evaluating cardiovascular biomarkers association to subsequent preeclampsia, SGA birth and combined disorders, we found different patterns.

First, preeclampsia was associated to several cardiovascular and inflammatory biomarkers, including elevated ACE2 and decreased levels of BNP. Notably, neither of these were associated with SGA birth. ACE2 is a key component of the renin-angiotensin system (RAAS), promoting vasoconstriction (176). It has previously been linked to especially preterm but also term preeclampsia by our group in a partly shared cohort. Furthermore, a previous study have reported elevated mid-pregnancy levels in women with subsequent SGA births, but decreased ACE2 levels at manifest preeclampsia regardless of SGA status when compared to normal pregnancies (177). However, their more inclusive definition of SGA birth (<10^{th} percentile) increases the risk of including fetuses without true growth restriction. Our findings contribute to current knowledge by observing elevated ACE2 levels in mid-pregnancy only in women with strictly validated subsequent preeclampsia but not SGA birth. This elevation could potentially contribute to an earlier rise in blood pressure during pregnancy.

BNP normally counteracts the RAAS to promote vasodilation (178, 179). BNP-elevation in the first trimester is generally suggested to reflect a normal physiological adaption to pregnancy-related volume expansion (180, 181). Conversely, we found that decreased BNP levels in mid-pregnancy were associated with subsequent preeclampsia. This finding aligns with results previously obtained by our group on a partly shared cohort, as well as with another separate study (171, 172). Our results broaden the existing evidence by observing no difference in BNP levels within the SGA group. These findings suggest an early cardiovascular imbalance in women who develop preeclampsia, which is absent or not as pronounced, in cases of isolated or combined preeclampsia and SGA birth.

Second, we found that subsequent SGA birth was associated with decreased levels of ADM, a peptide that regulates vascular tone, endothelial function, and has anti-inflammatory properties (182). Notably, ADM levels showed no association with preeclampsia. Our study adds to current knowledge on ADM, as there is only one previous study at time of birth showing elevated circulating ADM levels in women with FGR (n=19) undergoing caesarean section (183). Although this explorative study was restricted to 92 proteins, the overall differences in the number of dysregulated cardiovascular proteins suggests that the maternal cardiovascular system is less impaired in women with subsequent isolated SGA birth than in preeclampsia.

Although several proteins differed among the outcome groups, MMP-12 and PlGF, were the only biomarkers found to be dysregulated across all outcomes. These biomarkers have both vascular and placental features (166, 184-187). Their expression showed a greater deviation from controls in women with isolated SGA birth or combined preeclampsia and SGA birth than in those with preeclampsia alone. While PlGF has been extensively researched with similar results, studies on MMP-12 and preeclampsia or FGR are limited (133, 188, 189). MMP-12 is a proteolytic enzyme highly expressed by trophoblast cells and is upregulated by low oxygen in the first trimester (190). In normal pregnancy, MMP-12 levels typically decrease as placental blood flow and oxygen levels increases towards the second trimester (190). In women with subsequent preeclampsia decreased MMP-12 mRNA in first trimester placental tissue has been observed (170). Given that low oxygen and certain MMPs stimulate trophoblast invasiveness, it suggests that our findings on decreased MMP-12 in early pregnancy reflects mal-placentation in women with subsequent preeclampsia or FGR (170). Future basic research should further investigate MMP-12 changes across gestation and its role in trophoblast invasiveness during placental implantation.

These results suggest a broader cardiovascular impairment in women with subsequent preeclampsia than SGA birth, requiring further examination and validation. It is possible that measuring of cardiovascular biomarkers during the second trimester, when the cardiovascular load is increased, highlights these differences. Disadvantageous cardiovascular profile in early pregnancy, may not only disrupt placentation but also increase cardiovascular responsiveness to placental debris released due to syncytiotrophoblast stress (47). Future longitudinal studies should characterize the identified biomarkers throughout pregnancy. Further exploring a wider panel of cardiovascular biomarkers and further defining preeclampsia and SGA subtypes could add more knowledge on different cardiovascular profiles. Mapping such dysregulation will ultimately guide potential treatments aimed at stabilizing the cardiovascular system prior to or in early pregnancy.

Cardiovascular health after preeclampsia

Preeclampsia and long-term cardiovascular disease

Women who experience PIH, are at elevated risk of CVD (106). It remains unclear whether shared cardiovascular risk factors and latent CVD explain the elevated future CVD-risk, or whether preeclampsia itself introduces *de novo* risk through permanent cardiovascular damage.

To address this, we investigated multifetal pregnancies with preeclampsia, which often are excluded from research. Our findings show that, unlike singleton pregnancies, multifetal pregnancies with preeclampsia do not share a clearly elevated long-term CVD risk similar to that in normotensive singleton pregnancies. This finding partially aligns with one previous study, which, despite methodological differences (e.g., shorter follow-up, inclusion of both gestational hypertension and preeclampsia, and adjustment for pre-pregnancy risks), also found a modest increase in CVD risk after twin pregnancies with PIH, however still lower than that of singleton PIH (191). Given that a truly causal association would be expected to yield similar CVD risks regardless of fetal plurality, these results suggest that the link between preeclampsia and later CVD may not be directly causal.

Following our study, the short-term CVD risk after multifetal pregnancies with PIH have been studied with inconsistent results. One study report increased risk of hospitalisation due to CVD the year following a twin pregnancy regardless of PIH status, compared to normotensive singleton pregnancies (192). This could reflect the higher cardiovascular burden of a multi-fetal pregnancy, indicating that impaired recovery to baseline cardiovascular state postpartum may increase these women's vulnerability to CVD. They also found that cardiovascular mortality after PIH was higher among women with singleton pregnancies than those with twin pregnancies (192), which may indicate a higher range of latent CVD in the former group. However, one study found a threefold risk of CVD mortality during the first decade after a twin pregnancy with PIH, when compared to normotensive twin pregnancies. There was no difference in CVD mortality when comparing twin and singleton pregnancies complicated by PIH (193). However, this study did only adjust for maternal age at birth (193). Above studies vary in exposure, follow-up duration and adjustments for confounders, limiting comparability with our study. Nonetheless, some evidence suggests increased relative CVD risk in the first decade after PIH in multi-fetal pregnancies, which may originate in both subclinical CVD and cardiovascular damage due to PIH (194).

Theoretically, the large placental tissue in multi-fetal pregnancies may outgrow the uterine capacity with following hypoxia and angiogenic imbalance. The overall greater cardiovascular strain of multifetal pregnancy, and additional stress from this imbalance, may initiate a maternal endothelial response that culminates in preeclampsia. Since this transient pathway is driven primarily by the demands of the placenta, preeclampsia in multifetal pregnancies may not always reflect a pre-existing maternal cardiovascular vulnerability (34, 192). This origin contrasts with the likely latent CVD predisposition in singleton pregnancies with preeclampsia. In addition, while the combined strain of a multifetal pregnancy and PIH may impair cardiovascular recovery postpartum and elevate short-term CVD risk, the long-term outcomes appear unaffected, indicating no persistent cardiovascular damage or predisposing cardiovascular risk factors that would lead to CVD later in life.

The transient origin of preeclampsia in multifetal pregnancies, attributed to the placental burden and a high cardiovascular demand, is supported by a lower recurrence rate of PIH compared to singleton pregnancies. This suggests a cardiovascular predisposition only in the latter group (186). It may further be hypothesized that women with multi-fetal pregnancies who do not develop preeclampsia under the elevated burden, have particularly resilient cardiovascular systems, and therefore may carry a lower risk of CVD later in life (193). However, preeclampsia is a complex disorder with no distinct separation between different origins. Therefore, cardiovascular risk factors should be considered when assessing future CVD-risk in women following a multi-fetal pregnancy complicated by preeclampsia.

Cardiovascular risk factors after pregnancy induced hypertension

The association between PIH and elevated MI-risk is well established. However, the origin of this excess risk remains unclear. To further understand the association between PIH and the excess MI-risk in these women, cardiovascular risk profiles at first-time MI were assessed by prior PIH status.

In the years following PIH, the development and worsening of chronic hypertension and other cardiovascular risk factors is common (106, 195-197). However, previous studies on MI after a prior PIH are few, focus on MI before the age of 65, and are limited to small sample sizes. Further, in women with MI, the comparisons of risk factors between women with and without prior

PIH are limited and present conflicting results regarding prevalence of diabetes, dyslipidaemia, BMI and blood pressure (198-200).

In our study on women with first-time MI, those with prior PIH were younger and had higher blood pressure, even while on antihypertensive treatment, compared to those without PIH. Chronic hypertension was more prevalent in women with prior PIH, regardless of PIH subtype. This association was particularly strong in those with recurrent PIH. The direction of these findings is supported by a Norwegian study on cardiovascular risk factors. They found that the 41% of the increased MI risk in women with prior PIH, were mediated by diastolic blood pressure together with BMI (n=34) (115). Although, we found that antihypertensive treatment was more common in those with prior PIH, diastolic blood pressure levels remained significantly higher compared to those with prior normotensive pregnancies. These findings on cardiovascular risk factors at the first MI-event in women with prior PIH suggest a distinct underlying pathway to CVD. This pathway in women estimated to be at a low cardiovascular risk prior to pregnancy, may involve vascular damage and accelerated cardiovascular aging secondary to exposure to PIH (34, 106, 198, 201, 202). Such pathway would also align with results from a Swedish study on coronary atherosclerosis, showing increased risk of subclinical and obstructive artery disease in women with prior PIH compared to women with normotensive pregnancies (201).

In addition, in women with prior PIH without antihypertensive treatment at the MI-event, blood pressure levels were elevated and more often corresponded to a diagnosis of chronic hypertension than in those with prior normotensive pregnancies. Even though the blood pressures were registered in an emergency setting, the results may reflect on an underlying chronic hypertension (159).

Altogether, the results on women with or without antihypertensive treatment highlight underdiagnosis, undertreatment or resistance to treatment in women with prior PIH, at their first-time MI. Previous studies show that women with untreated chronic hypertension are more prone than men to develop left ventricular hypertrophy, which also is less responsive to antihypertensive therapy. In addition, they also face a higher risk of heart failure and increased arterial stiffness (203, 204). However, we found that women with prior PIH who were on antihypertensive treatment appeared to benefit significantly, experiencing a 5-year delay in their first MI compared to those without treatment. Antihypertensive treatment is previously established for prevention of MI (93, 203). In addition, timely postpartum blood pressure monitoring and treatment may support cardiac remodelling and reduce the risk of persistent left ventricular hypertrophy, which is strongly associated with later development of chronic hypertension (97). Therefore, early recognition and interventions in women with prior PIH could improve their future MI-risk.

Alongside above findings, women with prior PIH were more often obese, and had higher rates of ongoing antidiabetic and lipid-lowering treatment, compare to women with prior normotensive pregnancies. Among those without ongoing antidiabetic and lipid-lowering drug treatment, abnormal blood glucose and serum lipids levels were found. This is the first study to characterize these risk factors at the time of MI, strengthening previous evidence on that women with PIH are more likely to have or develop the metabolic syndrome, which is a major MI risk factor (34, 103).

We also found that smoking was substantially more common across our cohort of women with MI, than in the general population (204). However, smoking was more prevalent in women with prior normotensive pregnancies compared to those with prior PIH. This observation highlights the elevated risk of future MI among women with a history of PIH i.e., although they smoked less than women with prior normotensive pregnancies, tehy still had a higher risk of MI. This indicates the possibility of cardiovascular damage due to PIH markedly increasing their risk of CVD. The results also suggest that chronic hypertension and BMI in women with prior PIH, play a more dominant role in the development of MI, potentially outweighing the contribution of smoking.

The detailed data on cardiovascular risk factors at first-time MI assessed in this study, indicate that women with prior PIH face an elevated MI-risk, likely due to a poor cardiovascular risk profile dominated by unrecognized and undertreated chronic hypertension. Chronic hypertension, along with obesity which are closely associated, represents the main contributors of their increased MI-risk.

Currently, there is no consensus on how to monitor and follow-up cardiovascular risk factors after a pregnancy complicated by PIH. Implementing standardized follow-up of these factors could significantly impact the future risk of MI and enhance the long-term cardiovascular health in these women. However, their pathway to CVD may differ from the general population, partly due to possible vascular damage introduced during pregnancy. Therefore, future research should specifically assess the benefits of standardized monitoring and treatment response within this unique group.

Conclusion

Preeclampsia increase the risk of CVD, which remains a leading cause of mortality among women (205). This thesis, highlights the complex connection between the cardiovascular system, preeclampsia and future CVD.

Using a large-scale proteomic approach, several cardiovascular biomarkers with predictive potential were identified. Some biomarkers, including BNP, were specifically predictive for late-onset preeclampsia.

Women who developed preeclampsia showed extensive dysregulation of cardiovascular biomarkers, unlike those with subsequent SGA births. However, shared mechanisms were reflected by similar dysregulation of PlGF and MMP-12, both involved in placental and vascular functions.

In women with multi-fetal compared to singleton pregnancies, preeclampsia may originate from the increased cardiovascular burden rather than from underlying CVD. This is supported by the absence of increased long-term CVD-risk in this group.

Among women with prior PIH, chronic hypertension and obesity emerged as stronger risk factors for MI than smoking. Timely recognition and management of these conditions are therefore essential to reduce the MI-risk and improve long-term cardiovascular health in these women.

This thesis expands our knowledge on preeclampsia and related disorders through the lens of the cardiovascular system. It broadens the spectrum of potential predictive biomarkers and underscores distinct pathophysiological pathways leading to preeclampsia. Furthermore, it emphasizes improved postpartum cardiovascular risk assessment after pregnancies complicated by hypertension, to enhance long-term cardiovascular outcomes in these women.

Future perspectives

Currently there is an ongoing large case-cohort study using blood samples from the first trimester of pregnancy. In this study, on a heterogenous population, we will analyse absolute values of the potentially predictive biomarker MMP-12 for preeclampsia (identified in Paper I). This biomarker will be incorporated and evaluated as part of a predictive multivariate model.

In addition, studies on the expression on MMP-12 throughout pregnancy could improve knowledge on pathophysiological pathways to preeclampsia. These studies should define biomarker levels across different stages of pregnancy. Given the challenges in predicting late-onset preeclampsia, similar approaches could be useful regarding BNP, ACE2, and IDUA.

The role of MMP-12 in the placenta and its effects on the vasculature remain unclear. Further insight could enhance our understanding of the pathophysiological pathways and potentially contribute to preventive strategies or treatment. In vitro studies using human extravillous trophoblasts to analyse how MMP-12 affects their migration and invasiveness are suggested.

We further plan to evaluate the portion of the excess MI-risk that is mediated through chronic hypertension, by conducting a cohort study on chronic hypertension developed post-partum. In this study, we will include women without pre-gestational cardiovascular risk factors who have a singleton pregnancy with PIH, giving birth between 1973 and 2019. The exposure will be PIH during the first pregnancy. Follow-up will start 3 months post-partum. The outcome will be the first MI starting from 2005. The diagnosis chronic hypertension, will be captured by dispensed prescription of antihypertensive treatment.

There is an established consensus about optimised identification as well as treatment of chronic hypertension when home blood pressure measurement is applied (93, 206). Similar results have been observed in women in the antenatal and postpartum period with hypertensive disorders (92, 94-98). However, studies are limited and not evaluated in a Swedish context. A future study should assess home blood pressure measurement in women with PIH to evaluate whether maternal and neonatal outcomes are improved.

Sammanfattning på svenska

Preeklampsi (havandeskapsförgiftning) drabbar 2–8% av gravida kvinnor och innebär att den gravida utvecklar högt blodtryck (≥140/90) och tecken på organpåverkan efter den 20:e graviditetsveckan. Sjukdomen involverar flera organsystem och kan leda till allvarlig sjukdom och död både hos modern och fostret. Preeklampsi är även kopplat till negativa följder för kvinnans och barnets hälsa på lång sikt, t ex ökad risk för hjärt- och kärlsjukdom såsom hjärtinfarkt. För närvarande finns inget läkemedel som kan bota preeklampsi, utan förlossning är det enda sättet att stoppa sjukdomens förlopp.

Preeklampsi delas ofta upp i tidig och sent debuterande preeklampsi, med brytpunkt 34 graviditetsveckor. Tidig debuterande preeklampsi är oftare allvarligare och innebär ofta att barnet är tillväxthämmat och att en för tidig förlossning krävs. Sent debuterande preeklampsi är dock den vanligaste formen (ca 2,7% av gravida), och även dessa kvinnor kan drabbas av svåra komplikationer.

Riskfaktorer för att utveckla preeklampsi är idag välkända och innefattar bl a att kvinnan väntar första barnet, ärftlighet, övervikt, ålder>40, kronisk hypertension, diabetes mellitus och flerbördsgraviditet. Dessa riskfaktorer används ofta för att förutsäga vilka kvinnor som har en ökad risk att drabbas av preeklampsi. Tyvärr är träffsäkerheten låg (<40%). De senaste årtiondena har modeller för att förutsäga preeklampsi utvecklats och ofta ingår biomarkörer (proteiner). Den mest internationellt använda modellen har visat en bra träffsäkerhet gällande tidig preeklampsi (75%), men sämre gällande sen preeklampsi. Identifiering av nya biomarkörer skulle kunna öka träffsäkerheten.

Bakgrundsmekanismerna till preeklampsi är inte helt kända, men studier pekar på involvering av moderkakan och moderns hjärt- och kärlsystem. Graviditet i sig innebär stora fysiologiska anpassningar av hjärt- och kärlsystemet och otillräckliga anpassningar är kopplade till utveckling av preeklampsi, men även till andra komplikationer såsom tillväxthämning hos fostret. Den senare komplikationen är även den kopplad till nedsatt funktion hos moderkakan. Varför vissa graviditeter drabbas av preeklampsi och andra av tillväxthämning, är idag oklart.

Kvinnor som har haft preeklampsi har en minst fördubblad risk för hjärt- och kärlsjukdom, såsom hjärtinfarkt. Det saknas dock kunskap om detta gäller även kvinnor som haft preeklampsi i en flerbördsgraviditet. Dessutom vet vi inte vad som driver denna ökade risk för hjärt- och kärlsjukdom hos kvinnor med preeklampsi.

Det övergripande syftet med denna avhandling var att studera relationen mellan hjärt- och kärlsystemet och preeklampsi. Detta genom att studera hjärt- och kärlbiomarkörer i blodprov från ca 18 graviditetsveckor i syfte att dels finna nya biomarkörer för att förutsäga preeklampsi, dels studera deras uttryck beroende på om kvinnan utvecklat preeklampsi eller tillväxthämning. Dessutom, genom att använda nationella register studera relationen preeklampsi i flerbördsgraviditet och hjärt- och kärlsjukdom senare i livet, samt studera hjärt- och kärlriskfaktorer vid hjärtinfarkt hos kvinnor som tidigare haft preeklampsi eller annan graviditetsutlöst hypertoni under graviditet.

I den första och andra delstudien undersöktes 92 hjärt- och kärlbiomarkörer i plasma från cirka 18:e graviditetsveckan. I första delarbetet inkluderades 296 kvinnor som utvecklade preeklampsi, varav 30 stycken utvecklade den tidiga formen, samt 333 kvinnor som fortsatte vara friska under sin graviditet. Artificiell intelligens användes för att undersöka biomarkörernas förmåga att förutsäga preeklampsi. Vi fann flera biomarkörer som potentiellt skulle kunna bidra till framtida modeller för att förutsäga preeklampsi, bl a matrix metalloproteinas-12 (MMP-12) och natriuretisk peptid B (BNP). MMP-12 identifierade både de som utvecklade tidig och sen preeklampsi, medan BNP enbart identifierade sen preeklampsi.

I den andra delstudien undersöktes biomarkörernas uttryck hos kvinnor som senare utvecklade enbart preeklampsi, enbart tillväxthämning hos fostret eller båda diagnoserna. Studien inkluderade 273 kvinnor som utvecklade preeklampsi, 106 kvinnor som utvecklade ett tillväxthämmat foster och 31 kvinnor som utvecklade båda diagnoserna. Dessa grupper jämfördes mot 480 kvinnor som fortsatte vara friska under sin graviditet. Vi fann att enbart hos kvinnor som utvecklade preeklampsi skiljde sig ett flertal biomarkörer som speglar hjärt- och kärlsystemet. Däremot var uttrycket av MMP-12 liksom placentärt tillväxthormon (PlGF), vilka båda även är kopplade till moderkakan, avvikande hos alla fallgrupper. Mest avvikande var proteinerna hos de som utvecklade både preeklampsi och tillväxthämmat fostret.

I tredje delstudien undersökte vi risken att utveckla hjärt-och kärlsjukdom efter att ha genomgått en flerbördsgraviditet med preeklampsi. Studien innefattade 892 425 kvinnor som födde sitt första barn i Sverige mellan 1973 och 2010 och utvecklade en hjärtkärlsjukdom någon gång från tre månader efter förlossning till sista december 2011. Vi fann att kvinnor med flerbörd och preeklampsi i sin första graviditet inte hade en högre risk för hjärt- och kärlsjukdom jämfört med kvinnor som haft enkelbörd och normalt blodtryck under graviditet. Innebörden av detta kan vara att den bakomliggande orsaken till preeklampsi vid flerbördsgraviditet framför allt utgörs av den uttalade belastningen på hjärt- och kärlsystemet.

Fjärde delstudien undersökte hjärt- och kärlriskfaktorer hos kvinnor som insjuknar i sin första hjärtinfarkt. Kvinnorna jämfördes utifrån om de tidigare haft en graviditet där de utvecklat högt blodtryck (preeklampsi eller graviditetshypertoni) eller om de hade haft normalt blodtryck i alla graviditeter. Totalt studerades 17 814 kvinnor varav 1753 hade högt blodtryck under graviditet. Kvinnor som hade tillfälligt högt blodtryck under någon graviditet hade vid insjuknande i sin första hjärtinfarkt starkare koppling till att ha en behandling för kronisk hypertoni (högt blodtryck). Trots detta hade de ändå högre blodtryck och var oftare överviktiga. Däremot hade de svagare koppling till att vara rökare. Fynden tyder på att kronisk hypertoni utvecklat efter graviditet är en drivande orsak till att kvinnor som haft högt blodtryck under graviditet har en ökad risk att utveckla hjärtinfarkt senare i livet.

Sammanfattningsvis ger dessa studier stöd för att hjärt- och kärlbiomarkörer kan bidra till prediktion av preeklampsi, särskilt för preeklampsi med sen debut. Preeklampsi kan ha en starkare koppling till otillräckliga anpassningar i hjärt- och kärlsystemet än tillväxthämning av fostret har. Både preeklampsi och tillväxthämning av fostret ter sig dock vara kopplade till dålig funktion hos moderkakan. Dessutom såg vi en frånvaro av ökad risk för hjärt- och kärlsjukdom hos kvinnor som haft preeklampsi i flerbördsgraviditet, vilket talar för att mekanismerna bakom preeklampsiutveckling hos dessa kvinnor utgörs av den ökade belastningen på hjärt-och kärlsystemet. Vidare fann vi att efter genomgången preeklampsi är det viktigt att förebygga, upptäcka och behandla kronisk hypertoni som verkar vara en drivande orsak till ökad utvecklingen av hjärtinfarkt hos dessa kvinnor.

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