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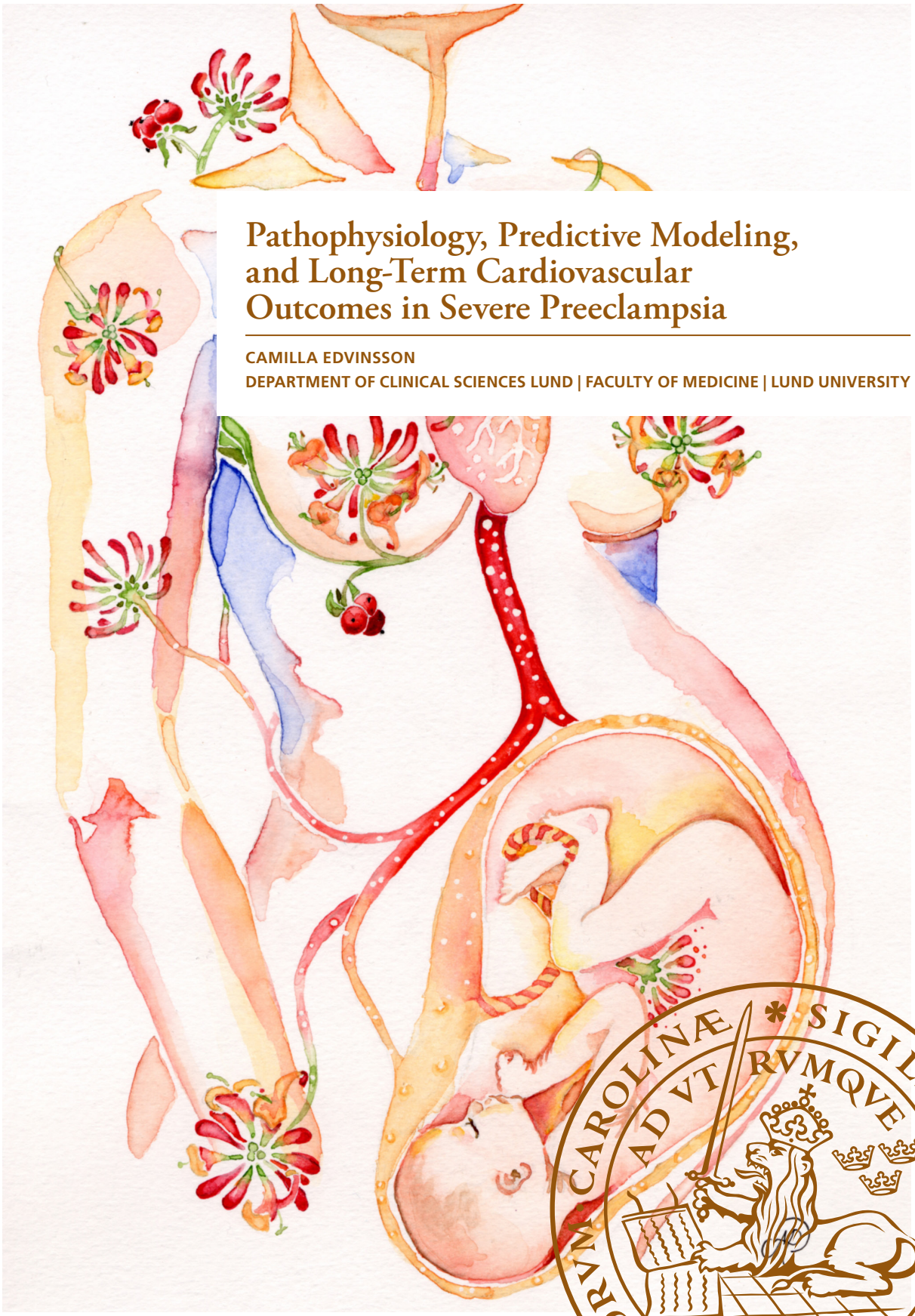

Pathophysiology, Predictive Modeling, and Long-Term Cardiovascular Outcomes in Severe Preeclampsia

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Camilla Edvinsson

LUND
UNIVERSITY

DOCTORAL DISSERTATION

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Abstract:

Preeclampsia (PE) is a pregnancy-specific disorder that is characterized by oxidative stress, angiogenic imbalance, and endothelial dysfunction leading to multiorgan manifestations. The resulting endothelial damage have a life-long impact on cardiovascular health, contributing to an increased risk of cardiovascular disease (CVD), stroke and diabetes.

The aims of this thesis were to describe the pathophysiology of severe PE by analyzing clinical risk factors and a subset of biomarkers, representing various signaling pathways described in PE. Another aim was to develop a predictive model for PE with need for intensive care and to evaluate the long-term postpartum impact of severe PE on the cardiovascular system.

In Paper I, biomarkers for oxidative stress, hemopexin (Hpx), alpha-1-microglobulin (A1M), and angiogenic imbalance, soluble fms-like tyrosine kinase-1 (sFlt-1), placental growth factor (PlGF), were analyzed in a cohort of intensive care patients with severe PE (n=41) versus PE patients not admitted to intensive care, PE controls (n=40), and normotensive pregnancies (n=40). In Paper III, markers for endothelial dysfunction, sphingosine-1-phosphate (S1P), hyaluronic acid (HA), syndecan-1 (SDC-1), heparan sulfate proteoglycan 2 (HPSG2), were analyzed in the same cohorts. The results showed that patients with severe PE had lower postpartum plasma concentrations of Hpx, A1M and S1P, and higher plasma concentrations of the glycocalyx degradation products SDC-1 and HA, indicating an impairment in the endogenous defense system against the oxidative stress in PE, in combination with signs of increased endothelial damage and vascular dysfunction. Correlation analysis revealed significant associations between analyzed biomarkers and physiological disease severity parameters such as blood pressure. Also, intensive care patients showed a postpartum sFlt-1:PlGF ratio in parity with antenatal levels indicative of adverse pregnancy outcomes previously described in PE.

In Paper II, the potential of using machine learning models to predict intensive care need in patients with preeclampsia was explored by incorporating numerical routine clinical and laboratory parameters from the intensive care cohort (n=41) and the PE controls (n=40). The variables that were most indicative of severe disease were aspartate aminotransferase, uric acid and body mass index, with an area under the curve (AUC) of 0.91. The model was validated internally on a test-set yielding a lower AUC of 0.85.

Paper IV comprised a prospective study that evaluated cardiovascular status in a selection of 10 patients from the intensive care cohort and 10 normotensive pregnancies, 4–7 years postpartum. Participants were examined with blood pressure measurements, echocardiography and cardiac magnetic resonance (CMR) imaging. Prior PE patients had a significantly higher E:A ratio (peak velocity blood flow during early filling versus peak velocity flow during atrial contraction) obtained by echocardiography. However no patient fullfilled the current clinical criterias for diastolic dysfunction. There was no difference in CMR-aquired parameters, indicating a normal heart morphology and function. The normal morphology was likely related to the lack of persistent hypertension in these cases.

Key words: preeclampsia, intensive care, biomarkers, machine learning, endothelial dysfunction, cardiovascular risk

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To my family

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List of papers

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

Paper I

Edvinsson C, Hansson E, Nielsen N, Erlandsson L, Hansson S R. Intensive care patients with preeclampsia – clinical risk factors and biomarkers for oxidative stress and angiogenic imbalance as discriminators for severe disease.
Pregnancy Hypertension: An International Journal of Women´s Cardiovascular Health 30 (2022) 88-94.

Paper II

Edvinsson C, Björnsson O, Erlandsson L, Hansson S R. Predicting intensive care need in women with preeclampsia using machine learning – a pilot study.
Hypertension in Pregnancy 2024, vol. 43, No. 1, 2312165.

Paper III

Edvinsson C, Piani F, Matthes F, Vanherle L, Erlandsson L, Meissner A, Hansson S R. Spinghosine-1-phosphate, a cardiovascular biomarker reflecting endothelial injury in preeclampsia.
Hypertension. 2025;82:00–00, published online Jan 2025, in print in the May issue on April 16th, 2025.

Paper IV

Edvinsson C, Steding-Ehrenborg K, Venkateshvaran A, Erlandsson L, Hansson S R, Hedström E. Women with severe preeclampsia with need of intensive care show no clear signs of cardiovascular disease five years postpartum.
Submitted March 2025.

Papers I-III are published open access. Paper IV is an unpublished manuscript.

Additional Work

Bucher V, Roddy Mitchell A, Gudmundsson P, Atkinson J, Wallin N, Asp J, Sennström M, Hildén K, **Edvinsson C**, Ek J, Hastie R, Cluver C, Bergman L. Prediction of adverse maternal and perinatal outcomes associated with pre-eclampsia and hypertensive disorders of pregnancy: a systematic review and meta-analysis. *EClinicalMedicine vol. 76 102861. 27 Sep. 2024.*

Jälmby M, **Edvinsson C**, Lykou D, Karampas G, Erlandsson L, Hansson S R*, Piani F*. Long-term microvascular and blood pressure dysregulation after Preeclampsia. *Accepted for publication in Hypertension Research, Feb 23rd, 2025.*

Abbreviations

AI	Artificial intelligence
A1M	Alpha-1-microglobulin
ACE	Angiotensin-converting enzyme
ACOG	American College of Obstetricians and Gynecologists
AKI	Acute Kidney Injury
ALAT	Alanine aminotransferase
ASAT	Aspartate aminotransferase
AUC	Area under the curve
BMI	Body mass index
CI	Confidence interval
CMR	Cardiovascular magnetic resonance (imaging)
CVD	Cardiovascular disease
CO	Cardiac output
EPE	Early-onset preeclampsia
FGR	Fetal growth restriction
GAG	Glycosaminoglycan
HA	Hyaluronic acid
HDL	High-density lipoprotein
HELLP	Hemolysis Elevated Liver enzymes, Low Platelets
Hpx	Hemopexin
HR	Heart rate
HSPG2	Heparan sulfate proteoglycan 2
HO-1	Heme oxygenase-1
ICU	Intensive care unit
ISSHP	International Society for the Study of Hypertension in Pregnancy
LDL	Low-density lipoprotein
LPE	Late-onset preeclampsia
LVM	Left ventricular mass

MAP	Mean arterial pressure
MeOH	Methanol
$MgSO_4$	Magnesium sulfate
NO	Nitric oxide
NOS	Nitric oxide synthase
PASIVA	Patient administrative system for intensive care units
PE	Preeclampsia
PI	Pulsatility index
PIERS	Preeclampsia Integrated Estimate of Risk score
PlGF	Placental growth factor
PWV	Pulse wave velocity
RAAS	Renin-angiotensin-aldosterone system
SDC	Syndecan-1
sFlt-1	Soluble fms-like tyrosine kinase 1
SIR	Swedish Intensive Care Registry
SOFA	Sequential Organ Failure Assessment
S1P	Sphingosine-1-phosphate
S1PR	Sphingosine-1-phosphate receptor
SphK	Sphingosine kinase
STB	Syncytiotrophoblast
STBEV	Syncytiotrophoblast extracellular vesicles
SV	Stroke volume
SVR	Systemic vascular resistance
T	Tesla
TNF-α	Tumour necrosis factor alpha

Preface

As a resident physician, I developed an early interest in obstetric anesthesiology – mostly because of the fascinating physiology that is observed in pregnancy, and the fact that we, as anesthesiologists, must treat 2 patients at the same time. Pregnancy renders anesthesia more dangerous, and depending on the gestational age, several considerations must be taken into account. In the first trimester, it is important to avoid drugs that can cause congenital anomalies, whereas in the third trimester general anesthesia is associated with a higher risk of airway problems and hypoxia especially during the induction of anesthesia.

After becoming a specialist, I had the opportunity to attend the 2-year Scandinavian Society of Anesthesiology and Intensive Care program in obstetric anesthesiology. This program comprised 5 international courses, a guideline task, and an individual research project. I had previously been hesitant about entering a PhD program due to the lack of projects that motivated me sufficiently to spend days, nights, and weekends working on them. However, this endeavor focused solely on obstetric anesthesiology, eliciting me in enthusiasm about conducting a research project and completing the program.

A colleague of mine in the intensive care unit suggested that I use the SWECRIT register and biobank – a project aimed at collecting blood samples from intensive care patients for analysis of various biomarkers. I began to consider the pregnant or newly delivered mothers who need intensive care. Compared with many parts of the world, Sweden has a low maternal mortality rate, but a risk of life-threatening situations remains during pregnancy, involving, for example, obstetric bleeding and preeclampsia. I have the greatest respect for both diagnoses, but the pathophysiology of preeclampsia puzzled me. The multiorgan involvement in this syndrome has much in common with sepsis – but entails a higher blood pressure instead of a lower one. However, many were the preeclamptic patients that had made me worried during my on-call days and nights.

After a search in the Patient Administrative System for Intensive Care Units (PASIVA), I identified a cohort of approximately 50 patients who had been cared for in the intensive care units throughout Skåne County due to complications of preeclampsia during the SWECRIT inclusion time. My next step was to find a supervisor who was willing to guide me through this project. I contacted an expert in preeclampsia, Professor Stefan R. Hansson, and booked a meeting from which the idea for this thesis eventually developed.

Introduction

Preeclampsia (PE) is a global disorder of pregnancy causing 76 000 deaths in women and 500 000 deaths in their children every year (World Health Organization). Despite decades of intensive research and the identification of several pathophysiological pathways that lead to PE, there is still no cure except for delivery of the fetus and placenta. The decision to deliver is often a balance between consideration of the mother´s state of health and the ability of the fetus to survive outside of the uterus, of which the mother`s well-being is prioritized.

Because PE is a heterogeneous disease with varying times of onset combined with multiple organ manifestations, it is difficult to predict the course of its clinical symptoms. Deterioration can occur quickly, and sometimes intensive care is needed to support organ functions and to save the life of the woman. In intensive care, it is possible to monitor the circulation invasively and administer intravenous drugs to regulate the blood pressure, treat respiratory insufficiency, neurological symptoms and kidney failure. Even after the woman has delivered, the syndrome does not resolve immediately, and dysregulated blood pressure can be challenging several days postpartum.

Preeclampsia affects the woman not only during pregnancy and in the peripartum period. A growing body of evidence supports the view that PE also increases the risk of developing cardiovascular disease (CVD) later in life and should be considered as an individual risk factor for the same. Although, this knowledge is widespread, there remains a lack implemented follow-up programs in Sweden to diagnose hypertension at an early stage – in addition to other cardiovascular changes – before the onset of clinical symptoms of manifest CVD.

The aim of this thesis was to describe the pathophysiology of severe PE that requires intensive care by analyzing clinical risk factors and a subset of biomarkers that correspond to various pathophysiological pathways that underlie the development of PE. Another aim was to develop a predictive model for severe PE with intensive care need using machine learning models based on routine laboratory parameters and clinical data. The last aim was to examine the presence of clinically significant CVD in a subgroup of intensive care patients 4 to 7 years after their index pregnancy.

Background

Preeclampsia

History

> "Pains have stopped; nothing will happen yet." (Sir Philip)
>
> "She is a healthy young woman going through a very normal and natural process." (Sir Philip)
>
> "Lady Sybil`s ankles are swollen; she seems muddled and not quite there, not quite in the present moment. It is my belief that Lady Sybil is at risk of eclampsia." (Dr. Clarkson)
>
> "A rare condition from which she is not suffering!" (Sir Philip)
>
> "Her baby is small; she is confused, and there is far too much albumin - that is protein in her urine." (Dr. Clarkson)
>
> "Lady Sybil is in distress; she is about to give birth!" (Sir Philip)
>
> "Lord Grantham, Mr Branson - time is running out; we should be at the hospital by now […]" (Dr. Clarkson)
>
> "But if she has the operation now, do you swear you can save her?" (Tom Branson)
>
> "I cannot swear it, but if we do not operate, and I am right about her condition, then she will die." (Dr. Clarkson)
>
> "If if if! Lord Grantham, can you please take command!" (Sir Philip)
>
> "Dr Clarkson is not sure he can save her. Sir Philip is certain he can bring her through it with a living child. Isn´t certainty stronger than a doubt?" (Lord Grantham)

The conversation above between Dr. Clarkson (a family doctor) and Sir Philip (an obstetrician) took place in Season 3 of the television series *Downton Abbey,* written by Julian Fellowes. Doctor Clarkson suspects that Lady Sybil is suffering from PE, with a risk of developing eclampsia, and thus recommends that she be taken

promptly to the hospital in order to end the pregnancy via an emergency Cesarean section. Sir Philip, however, wishes to calm the family down and diminish the impact of Dr. Clarkson's words. Shortly after this discussion, Lady Sybil gives birth to a healthy baby girl. She expresses happiness, but also fatigue and a desire to sleep. During the night, Lady Sybil develops seizures, eventually stops breathing, and dies, leaving her husband and the entire family in despair (not to mention the shock expressed by the dedicated audience of the series). This scene in Downton Abbey reflects what obstetric care could have been like in the 1920´s: a high mortality rate due to PE, but also a non-negligible mortality rate after a Cesarean section mostly due to infections [1].

Eclampsia, or convulsions during pregnancy and labour, was the first PE-related symptom that was documented, dating as far back as 3000 years B.C. [2, 3]. In Greek, eclampsia means "lightning" or "light burst", describing the unexpected onset of seizures as the feeling of being struck by lightning. At the end of the 17th century, it was known that primiparous women had an increased risk of developing eclampsia compared with multiparous women [3]. However, it was not until the 18th century that eclampsia was distinguished from epilepsy [2] and that it was recognized that delivery was essential for recovery from the syndrome [4]. The discovery of proteinuria in eclampsia – and in pregnancy that is not complicated by eclampsia – made the concept of pre-eclampsia known in the 19th century [3]. The invention of the inflatable arm-band in 1896 and the findings of hypertension in pregnancy, allowed the combination of increased blood pressure and proteinuria to be used as diagnostic criteria for PE [3]. Yet, opportunities for adequate treatment of symptoms remained non-existent.

Treatment modalities for PE have varied throughout history, including blood-letting, alterations in diet, and, of course, prayers [2]. At the start of the 20th century there were 2 main approaches to manage the syndrome. A more aggressive approach with induced delivery associated with a high mortality, and another expectant approach including large doses of morphine to treat convulsions while awaiting a natural delivery, which effected better outcomes, at least for the mother [2].

Returning to the compelling episode of Downton Abbey, delivery was natural and fast, and the only cure for PE to this date is delivery of the fetus and placenta. A visit to the hospital for an unnecessary Cesarean-section would likely not have had a positive impact on the outcome either. Eclampsia can still occur postpartum and without preceding hypertension. None of the characters can be blamed for failing to save Lady Sybil`s life. However, the obstetrician should have recognized the signs of PE. At the time, access to a sphygmomanometer was likely, perhaps allowing Lady Sybil`s blood pressure to have been discussed and considered in the diagnosis.

Definition

According to the International Society for the Study of Hypertension in Pregnancy (ISSHP, 2018), PE is defined as:

> Gestational hypertension accompanied by one or more of the following new-onset conditions at/or after 20 weeks of gestation:
>
> 1. Proteinuria
>
> 2. Other maternal organ dysfunction, including:
>
> - Acute kidney injury (AKI) (creatinine ≥90 μmol/L; 1 mg/dL)
> - Liver involvement (elevated transaminases e.g. alanine aminotranferase (ALAT) or aspartate aminotranferase (ASAT) >40 IU/L) with or without right upper quadrant or epigastric abdominal pain)
> - Neurological complications (e.g., eclampsia, altered mental status, blindness, stroke, clonus, severe headache, or persistent visual scotoma)
> - Hematological complications (thrombocytopenia – platelet count below 150,000/μL, disseminated intravascular coagulation (DIC), hemolysis)
>
> 3. Uteroplacental dysfunction (such as fetal growth restriction (FGR), abnormal umbilical artery Doppler wave form analysis, or stillbirth)

The definition of PE by the ISSHP, revised in 2018, differs from that of the American College of Obstetricians and Gynecologists (ACOG) from 2020, which states a lower platelet value of 100.000/μL and a slightly higher creatinine level of 1.1 mg/dL, compared with the criteria of the ISSHP (150.000/μL and 1 mg/dL). Also, the ACOG definition includes pulmonary edema, but uteroplacental dysfunction is not mentioned.

In Sweden, the Society for Obstetrics and Gynecology (SFOG), defines PE as a multiorgan syndrome with hypertension and new-onset organ dysfunction and/or uteroplacental dysfunction after gestational week 20. Kidney dysfunction is defined as creatinine ≥ 90 μmol/L or oliguria with a urine production of less than 500 mL per 24 hours. Coagulopathy is defined as rapidly declining platelet levels, or a platelet count of less than 100.000/μL. Pulmonary edema and fetal growth restriction (FGR) are included in the definition. The Swedish guidelines (SFOG) were revised in 2024 and provides the overall approach for the recommended management of PE in Sweden.

Hypertension is defined as a systolic blood pressure ≥140 mmHg and/or a diastolic blood pressure ≥90 mmHg [5]. However, the American Heart Association has changed its threshold for blood pressure to be considered as hypertension in non-pregnant individuals in 2017, to a systolic blood pressure of ≥130 mmHg and/or a diastolic blood pressure ≥80 mmHg. Before these values are adopted in pregnancy, the possible consequences for the fetus and mother must be evaluated further [6, 7], because lower blood pressure may lead to insufficient placental blood flow, especially if the placental vessels have an abnormal anatomy, as is commonly observed in PE.

There is no clear consensus on how to define severe PE – or more correctly, PE with severe features – but it is generally agreed that this diagnosis entails systolic blood pressure >160 mmHg and/or diastolic blood pressure >110 mmHg [8] and more severe organ involvement [9].

Proteinuria is no longer required for a diagnosis of PE, broadening its definition and explaining in part the rise in the incidence of PE in Sweden, per the Swedish Pregnancy Register.

The Hemolysis Elevated Liver enzymes, Low Platelets (HELLP) syndrome and general tonic-clonic seizures in pregnancy – i.e. eclampsia – are considered as severe forms of PE [8, 10].

Early-onset PE (EPE) is also viewed as a severe form of PE and is defined as PE that occurs before 34 weeks of gestation [8]. However, Roberts et al. opine that 37 gestational weeks is a more suitable point in pregnancy to delineate EPE, because of fewer fetuses are affected by growth restriction and because fewer placental vascular lesions are seen beyond this time [6].

Epidemiology and risk factors

Preeclampsia has a global prevalence of 2–6 % [6] and is a leading cause of maternal mortality in many parts of the world [11]. In Sweden, approximately 3 % of pregnancies are affected by PE, corresponding to 5000 cases per year. As discussed, these numbers are rising due to modifications to its diagnostic criteria, but may also be ascribed to a female population with increasing numbers of risk factors for PE. However, in recent years, maternal mortality due to PE has declined in Sweden. From 2011–2016, 10 fatal cases of PE were registered, decreasing to 1 in 2017–2022 (Swedish Pregnancy Register, Swedish Society of Obstetrics and Gynecology). This lower mortality may be attributed to an updated guideline for PE-care that were published in 2019. This new guideline was written in a multidisciplinary context through a collaboration between the Swedish Society of Obstetrics and Gynecology and the Swedish Society of Anesthesiology and Intensive Care Medicine. It guides the medical care of the PE patient – from the discovery of the syndrome in maternity care to the recommended, but insufficiently implemented, postpartum follow-up.

The prevalence of eclampsia has decreased successively in industrialized countries in modern times due to improvements in medical therapy with antihypertensive drugs and magnesium sulfate ($MgSO_4$) [9]. Eclampsia, however, remains a significant cause of maternal mortality in the developing world.

To prevent PE and identify those women who have a greater likelihood of developing the syndrome, awareness of the conditions and factors that increase the risk of PE is crucial.

Systemic disorders that are characterized by inflammation and oxidative stress, such as diabetes, kidney disease and CVD, increase the risk for PE, given their negative impact on the endothelium. It is believed that maternal risk elements interact with other factors, together contributing to the development of PE [12].

Age is a risk factor for PE – the prevalence of PE is significantly higher in women aged over 35 years compared with those aged under 20 years [9]. A family history of PE also increases the risk of developing the syndrome. Other risk factors include nulliparity, primipaternity, use of assisted reproductive technologies such as egg donation, and previous PE. However, primipaternity is debated as a risk factor, and several studies imply that adjusting for interpregnancy interval reduces the impact of this parameter [13]. Notably, a recently published study by Stenqvist et al. reported a heightened risk for PE associated with greater sperm deoxyribonucleic acid (DNA) damage, as measured by DNA fragmentation index [14] implicating a new paternal component of PE.

Several risk factors correlate with a predisposition for CVD in the mother, such as preexisting chronic hypertension, diabetes mellitus, obesity, obstructive sleep apnoea syndrome, and kidney disease [6, 11, 15].

Preexisting diabetes mellitus and gestational diabetes mellitus are associated with foremost late-onset PE (LPE) [16]. Hyperglycaemia in dysregulated diabetes causes endothelial damage and systemic inflammation, whereas well-regulated diabetes reduces the risk of PE. It is not established whether metformin has a clear advantage over insulin in the treatment of diabetes mellitus during pregnancy and regarding the risk of developing hypertensive disorders of pregnancy [17, 18]. An ongoing international study (PI4) by Bergman et al. is investigating whether metformin prolongs pregnancy that is already affected by PE (ClinicalTrials.gov ID NCT06033131). A previous pilot study has generated promising results with extending pregnancy by approximately 1 week, which may have a positive impact on neonatal outcomes [19, 20].

Autoimmune disorders, such as systemic lupus erythematosus (SLE) and the antiphospholipid syndrome are well-known risk factors for PE, whereas hypothyroidism, for example, requires further study to be established as a risk factor [21]. Another factor that needs further evaluation is fetal sex, wherein the presence of a male fetus may be associated with an increased risk of LPE [22].

Infections, such as periodontal disease can lead to systemic inflammation via bacteriemia which not only is a risk factor PE, but also the development of CVD and atherosclerosis [23].

During the Covid-19 pandemic, it was discovered that the coronavirus exploited the angiotensin converting enzyme (ACE) 2 receptor, expressed on endothelial cells, causing endothelial inflammation and eliciting PE-like symptoms [24] by inhibiting the hydrolysis of angiotensin II. Covid-19 also increased the risk of PE development in a dose-dependent manner, wherein more severe Covid was associated with a greater risk of PE with severe features [25].

Changes in the gut flora have been proposed to increase the risk of developing PE. The bacterial profiles in fecal samples from PE patients differ from those of healthy pregnancies. Diversity in bacterial composition may affect permeability in the intestines and induce inflammation [26], however, the exact pathophysiological mechanisms that lead to PE are unknown. Result from an animal study showed that transferring faecal flora from humans or mice with PE to normotensive control mice induced PE symptoms [27]. Whether alternations in bacterial composition lead to inflammation or whether systemic inflammation affects the intestines needs to be determined. To seek further knowledge, an ongoing randomized trial by Meijer et al. is studying whether the addition of probiotics reduces the risk of PE in a pregnant population (ClinicalTrials.gov ID NCT06700044).

Clinical risk factors for PE	
High risk	Autoimmune diseases, such as SLE Previous PE or eclampsia Previous gestational hypertension or intrauterine growth restriction Diabetes type 1 or 2 Multiple births Renal disease Chronic hypertension IVF with egg donation
Moderate risk	Nulliparity Heredity for PE Body mass index > 30 Age > 35 (40) Pregnancy intervals > 10 years African origin Verified obstructive sleep apnea

Pathophysiology

As discussed, PE can be divided into EPE and LPE, of which EPE is caused primarily by defective placentation. Late-onset PE depends more on maternal factors and the senescence of the placenta at the end of pregnancy. Both types, however, are characterized by syncytiotrophoblast (STB) stress, causing endothelial cell activation and systemic inflammation, manifesting clinically as varying degrees

of maternal end-organ damage [28]. The syncytiotrophoblast constitute the outer layer of the trophoblast that invades the uterus and form the barrier between the fetus and the pregnant woman, exchanging gases and nutrients between them and producing hormones to maintain the pregnancy.

The 2-stage theory of the development of PE is the most established model, in which the first stage consists of inadequate placentation and the second stage is characterized by the clinical symptoms that arise as a consequence of the insufficient uteroplacental perfusion [12, 29, 30]. The spiral arteries are central in the theory of defective placentation.

Establishment of the maternal-fetal circulation in pregnancy, requires invasion of the maternal endometrium by the fetal trophoblast [31]. According to Redman et al., the spiral arteries are initially plugged by intraluminal extravillous trophoblasts and the oxygen tension in the placenta is low. These trophoblasts contribute to the remodeling of the spiral arteries by replacing endothelial and smooth muscle cells to reduce the vascular resistance (**Figure 1A and 1B**). In PE, invasion by extravillous trophoblasts is insufficient impairing remodeling [32, 33]. This insufficient remodeling of the spiral arteries leads to narrower vessels with a more pulsatile flow at a higher pressure [30]. The retention of muscle seen in the media wall renders the spiral arteries more reactive to substances that cause vasoconstriction [23]. Insufficiently remodeled vessels are also more prone to developing atherosis – atherosclerosis that is specific to the spiral arteries [23]. In contrast, in normal placental development with adequately developed spiral arteries, the low-pressure system allows for even blood flow, adequate oxygenation and delivery of nutrients. The divergent placental flow in PE can be reflected as a higher pulsatile index (PI) by Doppler technique. When examining placentas from EPE after delivery, it is more common to see ischemic areas with spiral artery thrombosis leading to infarction compared with placentas from LPE [23, 30].

The placental dysfunction seen in LPE is instead a consequence of an aging placenta due to placental stressors, resulting in a dysfunctional placenta that is unable to withstand the increasing demands of pregnancy, in conjunction with maternal risk factors – e.g. preexisting systemic diseases. Even in normal pregnancy, the circulation to the placenta declines at approximately 30 weeks of gestation due to increased resistance, given the limited space for the placenta in the uterus – i.e., ”the small pot theory”. This lack of space leads to progressive intraplacental hypoxia, which may effect PE. In fact, in an unpublished study, Lykou et al. noted an angiogenic imbalance that approximates PE in prolonged pregnancies that are otherwise considered as normal (gestation week 42).

The reduced perfusion and placental hypoxia in both types of PE cause STB stress, which manifests as internal cellular oxidative stress, apoptosis, and the release of STB-derived extracellular vesicles (STBEVs) into the maternal circulation (**Figure 2**) [12, 34]. These vesicles contain substances that elicit the systemic inflammatory stress syndrome, endothelial damage, oxidative stress, vasoconstriction and antiangiogenic-activity in the pregnant woman, resulting in

multiorgan manifestations (**Figure 3**). Syncytiotrophoblast-derived vesicles in PE cause significantly stronger angiotensin II-mediated contractions and structural damage to human subcutaneous arteries than vesicles from normal pregnancy; these effects can be blocked by using chlorpromazine or a receptor-specific antibody [34].

As discussed, PE is characterized by angiogenic imbalance. The placenta normally produces the anti-angiogenic factor soluble fms-like tyrosine kinase-1 (sFlt-1) and the angiogenic factor placental growth factor (PlGF). As the pregnancy progresses, sFlt-1 predominates over PlGF, and the ratio between them is further skewed in PE [30]. Soluble fms-like tyrosine kinase-1 has a negative impact on the endothelium and its function, which will be expounded on in the chapter on angiogenic imbalance and in the section on endothelial injury and the glycocalyx.

Pregnancy is normally associated with a moderate degree of inflammation, but the systemic inflammatory response in PE is significantly more pronounced, manifesting as higher levels of pro-inflammatory interleukins and tumour necrosis factor alpha (TNF-α). In fact, the maternal immune system already appears to be dysregulated early in pregnancies that later develop PE. This maladaptive immune response, with its lowered fetal tolerance, seems to affect placental development [32]. The HELLP-syndrome, a severe form of PE, entails a systemic inflammatory response that resembles the immune response that arises in sepsis. Treatment with $MgSO_4$ in eclampsia has an immunomodulatory effect and may dampen the inflammatory response in PE by reducing the levels of circulating inflammatory markers [35].

Another complicating element in the pathophysiology of PE is that maternal cardiovascular dysfunction may precede the development of PE and thus be the causative factor of relative hypoperfusion in maternal organs, including the placenta [36]. This topic will be discussed in the following chapter on the cardiovascular system.

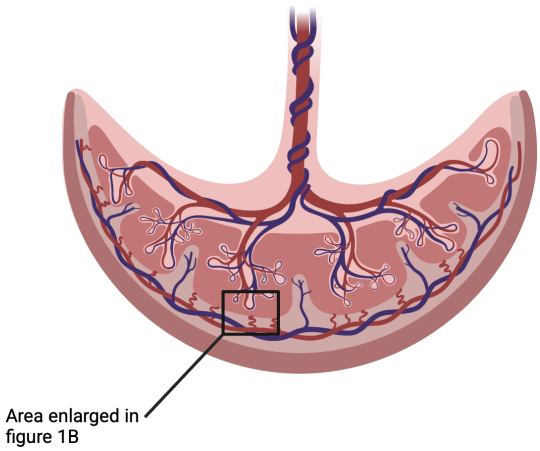

Figure 1A
Human placenta, showing the location of the chorionic or placental villus and corresponding maternal endometrium with spiral arteries within the square, marking the area enlarged in figure 1B. Figure created in Biorender.com.

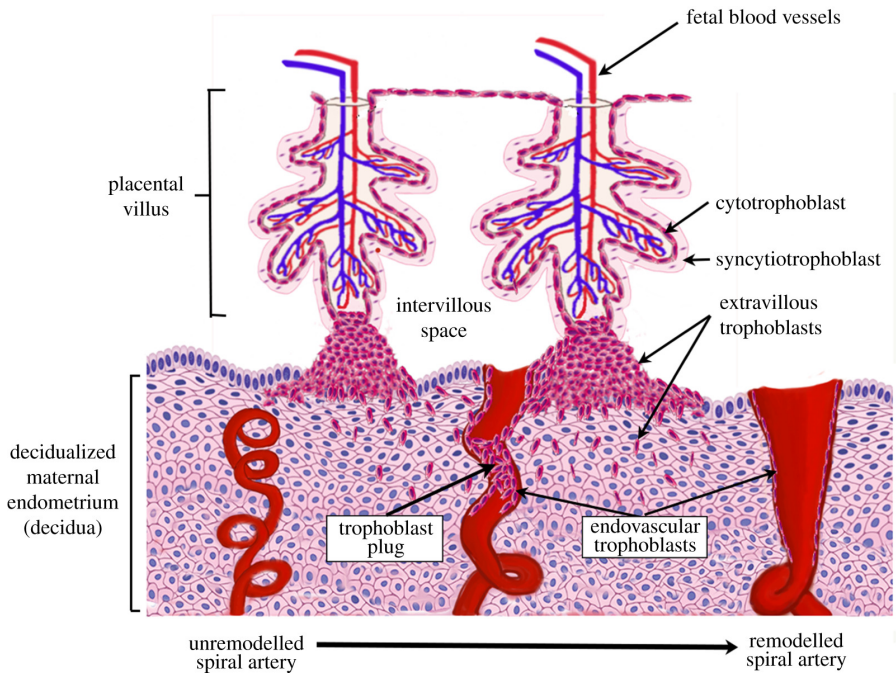

Figure 1B
Schematic of the chorionic or placental villus with the syncytiotrophoblast and cytotrophoblast. The figure also illustrates the path from unremodelled spiral arteries to the remodelled arteries with the potential for low-pressure perfusion. The figure is reprinted with permission from Saghian et al. [37].

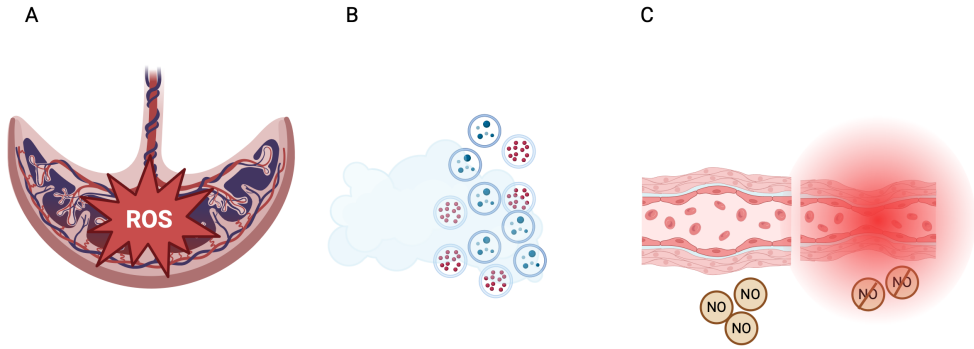

Figure 2
The hypoxic placenta is illustrated to the left (A). Oxidative stress in the placenta leads to STB-stress and the release of STB-derived vesicles (B). The vesicles contain substances leading to vascular inflammation, endothelial dysfunction and vasocontriction through downregulation of nitric oxide pathways (C). ROS – reactive oxygen species, NO – nitric oxide. Figure created in Biorender.com.

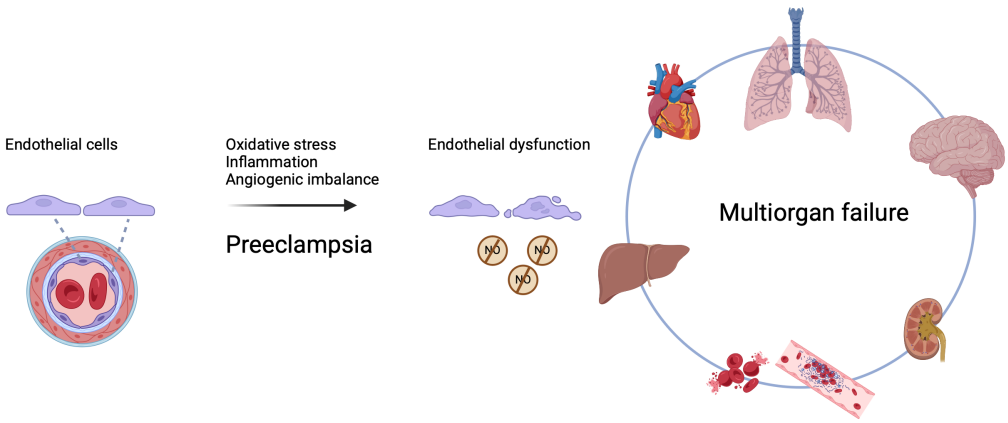

Figure 3
Endothelial cells lining the inner surface of the blood vessels. The endothelial dysfunction caused by oxidative stress, inflammation, angiogenic imbalance and STB-vesicles in PE, leads to multiorgan involvement and in severe cases multiorgan failure. Figure created in Biorender.com.

The cardiovascular system

Cardiovascular adaptations in normal pregnancy

Pregnancy is sometimes referred to as “the ultimate stress-test” of the female cardiovascular system, with the potential to reveal previously asymptomatic cardiac pathology. Normal pregnancy is associated with vasodilatation leading to a decrease in systemic vascular resistance (SVR), caused by the hormones estrogen,

progesterone and relaxin [38]. Systemic vascular resistance reaches its lowest value during the second trimester, increases modestly as the pregnancy progresses, and returns to near-prepregnancy levels postpartum [39, 40]. Blood pressure lowers due to this vasodilatation, with a greater reduction of diastolic pressure compared with systolic pressure. Patients with a higher body mass index (BMI), experience a smaller reduction in blood pressure [41]. The reasons for this are currently unknown, but the higher inflammatory status in obesity could have an impact on blood pressure levels [42].
During normal pregnancy, sympathetic activity rises, as does activation of the renin-angiotensin-aldosterone system (RAAS), but the receptor response to vasoconstrictors such as angiotensin II [43], attenuates. However, the increased activation of the RAAS in which the levels of angiotensinogen and angiotensin II climb, leads to retention of sodium and water [44]. The consequent increase in intravascular volume counteracts the effects of vasodilatation on blood pressure. Despite the greater reabsorption of sodium, water retention will dominate, lowering plasma osmolality and thus resulting in the hyponatraemic hypervolemia that is seen in normotensive pregnancies [45]. Cardiac output (CO) rises due to an increase in stroke volume (SV) and heart rate (HR), peaking by the end of the second trimester [46]. The higher plasma volume also increases cardiac left ventricular mass (LVM). The remodeling of the heart in normal pregnancy is called “eccentric” remodeling, in contrast to the “concentric” remodeling that is observed in PE. In the latter, wall thickness increases without a simultaneous rise in ventricular dimensions [47].

Cardiovascular maladaptations in preeclampsia

Early-onset and late-onset PE are underpinned by disparate pathophysiological pathways but also with regard to their impact on the cardiovascular system. Hypertension, however, is part of the definition of PE and is common to both of its forms. Mean arterial pressure (MAP) is the most reliable measure of perfusion [48] and is calculated by:

MAP = CO x SVR

(Ohm´s law)

If a rise in blood pressure is caused primarily by an increase in SVR, it is categorized as “resistance-dominant” hypertension, but if the increase in CO is the most prominent factor, the hypertension is classified as “volume-dominant” [49, 50].

The placenta-derived factors that are released into the maternal circulation in PE lead to inflammation, endothelial damage and a reduction in vasodilators such as nitric oxide (NO), and prostacyclin, whereas the vasoconstrictors endothelin-1 and thromboxane A2 are upregulated [51]. These changes parallel a rise in sensitivity to angiotensin II, another potent vasoconstrictor [34] that increases endothelin-1

production [52]. In particular, a reduction in NO is a common outcome of several pathophysiological pathways and is pathognomonic of the endothelial dysfunction in PE.

Early-onset PE is characterized by hypertension, increased SVR and a decrease in CO, often in combination with fetal growth restriction (FGR) [53]. The increase in SVR leads to a compensatory decline in intravascular volume, hemoconcentration and a lower tolerance to bleeding/fluid loss. Another theory regarding why intravascular volume decreases is plasma leakage due to disruptions in the endothelial barrier, leading to interstitial edema. Loss of intravascular volume leads to subsequent failure to increase CO [54]. A growing body of evidence suggests that the cardiac changes that are observed in EPE may be attributed to preexisting cardiac pathology that can already be measured before conception [55, 56].

Conversely, LPE has been proposed to be divided further into 2 types [49, 50] (**Figure 4**). Type I entails an increase in CO and low SVR, wherein the CO and volume expansion are greater than in normal pregnancy, explaining the rise in blood pressure. In type II, this increase in plasma expansion, in combination with higher abdominal pressure due to the pregnancy, causes shear stress, especially in the venous system, leading to disturbed relaxation, venous dysfunction and organ congestion. These effects manifest as a sudden third trimester change from low to high SVR in combination with reduced CO [50]. Hypervolemia causes shear stress, shedding of endothelial glycocalyx, and vascular dysfunction/stiffness, which could impact the circulatory transition that has been described above [57]. The endothelial glycocalyx will be discussed in the next section.

Gestational hypertension and PE have been proposed to differ based on an increased inflammatory response in PE in combination with dysfunctional venous hemodynamics as explained earlier [49, 58, 59].

The various cardiovascular types in PE are important to consider when treating the high blood pressure. For example, in EPE, or in LPE with resistance-dominant hypertension, the use of betablockers (such as labetalol) can have an additional negative impact on the reduced CO, whereas treatment with dilatating calcium-blockers is less suitable in PE with an already low SVR [50, 60, 61]. Transthoracic echocardiography is recommended in Swedish PE guidelines to evaluate cardiac anatomy and function for not only suspected heart failure, but also in severe PE for individualized treatment (SFOG).

In contrast to normal cardiovascular adaptations to pregnancy, there is pathological ventricular remodeling in PE – described as concentric hypertrophy [47]. In a study on PE at term, 20 % of the patients showed more pronounced remodeling leading to diastolic dysfunction [62]. These women may be at a greater risk for pulmonary edema during pregnancy and, more importantly, at delivery due to the volume load via autotransfusion from the uterus. Pronounced cardiac remodeling in PE has also been speculated to increase the risk of future CVD, due to irreversible myocardial damage [62].

The fact that PE is less common in multiparous compared to nulliparous women could be attributed to the normal cardiac protective remodeling that has already occurred in previous pregnancies – referred to as pre-conditioning [63]. As a result, the stress-test of pregnancy has already been endured in these women.

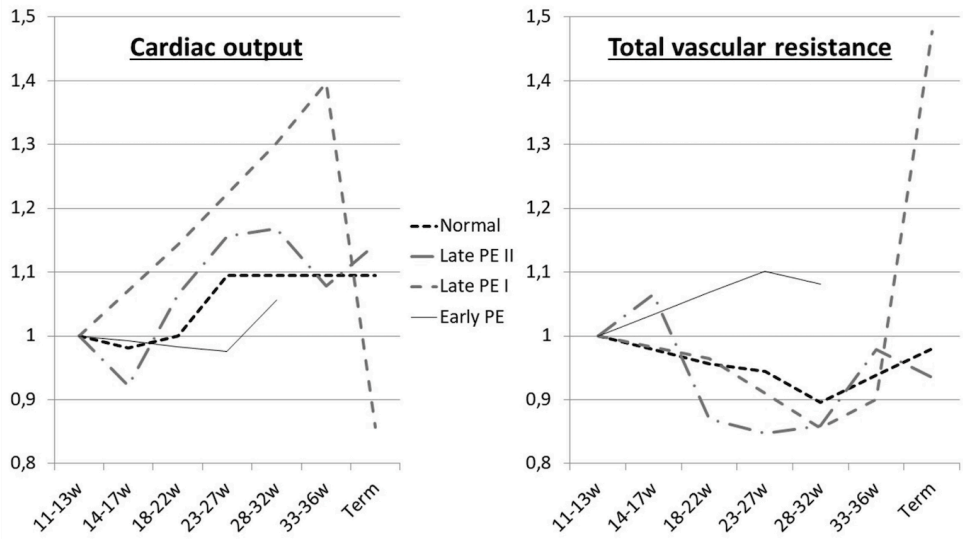

Figure 4
Various cardiovascular phenotypes in EPE and LPE in relation to gestational week. Re-printed with permission from Masini et al. [50].

Endothelial injury and the glycocalyx

The endothelial glycocalyx (**Figure 5**) is a negatively charged mesh-like layer that covers the vascular endothelium and mediates several physiological processes. It regulates vascular permeability, vascular tone, and interactions between vessels and circulating blood cells; has antithrombotic properties, governs angiogenesis, and inflammatory responses; and protects the vessel wall from shear stress [57, 64-66].

The endothelial glycocalyx comprises a membrane-bound component that is composed of proteoglycans, glycoproteins, and glycosaminoglycans (GAGs) and a soluble element that is made up of plasma proteins and GAGs [64, 65]. Proteoglycans are a defined subclass of glycoproteins and consists of a core protein of syndecans and glypicans, to which GAG chains are linked. Heparan sulfate and hyaluronic acid (HA) are 2 examples of GAGs [64]. The most common proteoglycan in the glycocalyx is heparan sulfate proteoglycan (HSPG) consisting of heparan sulfate that is bound to syndecan. Hyaluronic acid is important for stabilizing the glycocalyx but differs from heparan sulfate by not being bound to a

core protein. Soluble components such as albumin and orosomucoid also help preserve the endothelial glycocalyx as a permeability barrier [64].

The endothelial glycocalyx can be damaged by enzymes, pro-inflammatory cytokines, ischemia and reperfusion – which causes degradation or shedding of the glycocalyx – and disruption of the endothelial barrier [64, 65]. Increased plasma levels of glycocalyx degradation products have been observed in sepsis, correlating to the degree of organ failure [67] and in trauma, associated with the extent of capillary permeability [68].

Shear stress of the glycocalyx induces NO-production in endothelial cells, leading to vasodilation. It has been hypothesized that dysfunction of the glycocalyx constitutes the first step in the development of arteriosclerosis [69].

The angiogenic imbalance in PE – comprising an overweight of placental production of sFlt-1 – leads to collapse of the glycocalyx due to the binding of sFlt-1 to heparan sulfate. This deterioration of the glycocalyx layer leads to vascular stiffness and leukocyte adhesion, further causing tissue injury and organ dysfunction [70]. Notably, Schulz et al. showed that heparin protects against sFlt-1-induced glycocalyx damage [70].

Several studies have reported derangement and shedding of the glycocalyx in pregnancy and PE. Immonen et al. observed higher levels of syndecan-1 (SDC-1) in pregnancy with plasma concentrations increasing with gestational age. This upregulation was hypothesized to have been driven by the release of SDC-1 from the STB glycocalyx in conjunction with increased endothelial glycocalyx shedding due to the inflammatory state in normal pregnancy. Syndecan-1 levels were even higher in pregnant patients with pyelonephritis, which often is associated with bacteriemia and further inflammation [71]. Notably, the STB layer in the placenta also contains a glycocalyx, but lacks HA and heparan sulfate, while it can produce SDC-1. However, in PE and FGR, a lower SDC-1 expression has been found on the STB, instead supporting the hypothesis that the higher levels of SDC-1 in the maternal circulation in PE do not originate from the placenta, but most likely stem from maternal plasma as a result of vascular endothelial damage [72].

In HELLP syndrome, a severe form of PE that entails multiorgan involvement, plasma levels of SDC-1 are elevated, correlating with its clinical course [73].

Increased plasma levels of SDC-1, HA, and heparan sulfate proteoglycan 2 (HSPG2) have been observed in EPE, whereas in LPE such levels were significantly higher only for HA. The elevated plasma levels of glycocalyx degradation products correlate with cardiovascular changes, such as arterial stiffness [72]. The higher plasma levels of HA, in EPE and LPE has also been reported by Kornacki et al., confirming indirect signs of endothelial damage in both types of PE [74].

It is also possible to measure the real-time thickness of the glycocalyx by using sublingual capillaroscopy. Weissgerber et al. noted a reduction in glycocalyx thickness in EPE, as evidenced by a larger perfused boundary region in the blood vessels. This change correlated with significantly higher amounts of glycocalyx degradation products in plasma [75].

Of the functions of the endothelial glycocalyx that have been discussed, all are affected to varying extents in PE as a sign of impairment of this protective layer. Such examples of important organ manifestations include increased endothelial permeability leading to tissue edema; reduced endothelial antithrombotic function effecting intravascular coagulation; and an overload of vasoconstrictors causing hypertension [76]. Preexisting kidney disease is associated with glycocalyx degradation, and kidney failure in PE is also related to endothelial damage and podocyte loss [77].

There is no established medical treatment to maintain or regenerate the endothelial glycocalyx. Albumin and fresh frozen plasma have been shown to restore the glycocalyx in animal models after massive bleeding and sepsis [67, 76, 78]. Sphingosine-1-phosphate (S1P), a sphingolipid that is bound to plasma albumin, and high-density lipoprotein (HDL) also have protective effects, preserving the endothelial glycocalyx and limiting shedding [57]. The association between S1P, PE, and the endothelial glycocalyx will be discussed further in the section on S1P.

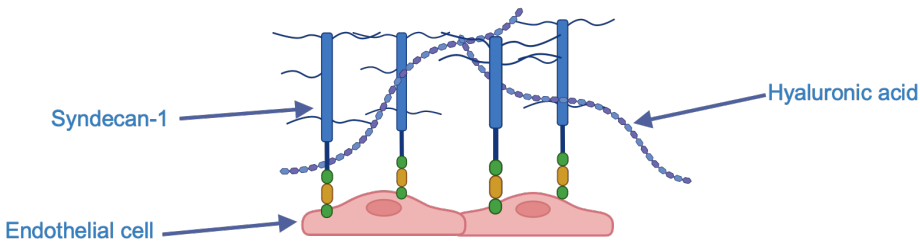

Figure 5
Endothelial cells covered by a glycocalyx layer. This layer consists of proteoglycans and glycosaminoglycans such as syndecan-1 (shown with heparan sulfate attached) and hyaluronic acid. Figure created in Biorender.com.

Cardiac changes after preeclampsia

There is a well-established link between previous PE and future CVD, although the exact cellular mechanisms that underlie the long-term risks of PE remain incompletely understood [79-81]. The American Heart Association has categorized PE as an independent female-specific cardiovascular risk factor. Further, PE is the factor that brings the CVD risk for women closer to the risk for men [82]. Recurrent PE increases the cardiovascular risk further through repetitive impact on the cardiovascular system and may shorten the time interval from pregnancy to the development of clinical symptoms [83]. After a pregnancy that has been complicated by PE, women have a greater risk of developing chronic hypertension – especially for preterm PE, wherein 40 % of such pregnancies develop hypertension within 1-2 years postpartum [36, 84]. Women with a history of PE are

also at increased risk of early vascular aging defined as higher-than-predicted aortic stiffness, based on blood pressure data and age [85, 86]. Furthermore, women with severe PE, EPE and recurrent PE are considered as high-risk groups for early vascular aging [85]. Women with previous PE who developed hypertension have increased arterial stiffness, by applanation tonometry, compared with previous PE without hypertension 6 months to 6 years postpartum [87]. However, prior PE patients without hypertension also showed signs of affected arterial health manifesting as higher central blood pressure and higher levels of low-density lipoprotein (LDL), versus healthy controls [87]. Paquin et al. concludes that there is a gradual vascular change from healthy pregnancy to PE without hypertension and, finally, to PE with persistent hypertension. Women with previous PE, independent of blood pressure, also had a higher BMI and hip-to-waist ratio than healthy controls in the studies above [87].

In a cross-sectional study, based on female participants in the Swedish Cardiopulmonary Bioimage Study (SCAPIS), a significant association was observed between a history of hypertensive disorders in pregnancy and coronary artery disease, despite the women with coronary atherosclerosis being considered otherwise to have a low cardiovascular risk [88]. In a similar study of younger women aged 40-55 years, comprising former PE women and controls, by computed cardiac coronary tomography, PE persisted as an independent cardiovascular risk factor for coronary atherosclerosis, after adjustments for confounding factors [89].

Oxidative stress in preeclampsia

Oxidative stress is defined as a state in which the excessive production of oxidative reactive species overwhelms the endogenous anti-oxidative defense system, leading to tissue damage [90, 91]. Normal pregnancy entails an imbalance between pro-oxidants and anti-oxidants, manifesting as rising levels of oxidized-LDL and decreased antioxidant capacity [92]. The increased oxidative stress in normal pregnancy is believed to be related to the systemic inflammatory response – a change that is even more pronounced in PE [91, 93, 94].

There are 2 main types of oxidative stress in PE: oxidative stress that arises in the defective placenta, and maternal oxidative stress, which is induced by factors from the placenta.

The placenta develops initially in a hypoxic environment, and the insufficient remodeling of the spiral arteries in EPE leads to uneven blood flow, with intermittent hypoxia and reperfusion. These repeated episodes of hypoxia-reoxygenation induce oxidative stress and, foremost STB stress. Thus, alternating incidents of hypoxia and reperfusion constitutes a greater cause of oxidative stress than hypoxia alone [90]. Oxidative stress in the placenta can cause cell damage, apoptosis, and cell senescence [12]. The stressed STB also sheds placental STBEVs,

the levels of which correlate with maternal blood pressure [95]. This correlation with clinical disease severity markers such as blood pressure, can be interpreted as the more toxic factors delivered into the maternal circulation affecting the endothelium, the more severe form of PE.

One theory of the cause of oxidative stress in PE is the release of heme into the maternal circulation. Masoumi et al. showed that in an induced hypoxic environment STBs express α-globin protein [96], a subunit of hemoglobin, and a component of both fetal and adult hemoglobin. Each subunit of hemoglobin carries an iron-molecule, or heme group.

It has been indicated [97] – but not proven – that STBEVs containing both fetal hemoglobin and α-globin, are shed from the placenta to the maternal blood stream and taken up by the maternal endothelium, inducing cellular damage and vasoconstriction. As discussed, vascular tone is regulated by endothelial nitric oxide synthase (NOS). Notably, α-globin is expressed in arterial endothelial cells, in which it regulates vasodilatation and vasoconstriction through the endothelial NOS pathway. Alpha-globin may thus contribute to the maternal hypertension in PE by binding NO to heme [98, 99]. The downregulation of NO is characteristic of the endothelial dysfunction in oxidative stress.

Placental hypoxia may also be worsened by elevated levels of free fetal hemoglobin that originates from the fetus, as seen in cord blood in FGR, in which an excess of free fetal hemoglobin increases fetal-placental vascular resistance by binding of NO [100]. Higher levels of free fetal hemoglobin in FGR fetuses likely originates from hemolysis, in combination with an insufficient heme scavenging system, manifesting as lower levels of hemopexin (Hpx) [100]. Hemopexin will be discussed below.

Another noteworthy aspect is that iron metabolism as a whole appears to be affected in PE, wherein ferritin concentrations rise in the first trimester of women who later developed LPE [101]. Higher ferritin levels can also be attributed to inflammation, as observed in several inflammatory diseases, rendering it an unspecific marker. Iron-metabolism is regulated by erythroferrone and hepcidin. Hepcidin lowers the availability of iron, and erythroferrone increases plasma iron availability by suppressing hepcidin, potentially leading to iron overload in, for example, PE pregnancies [95]. Higher levels of erythroferrone have been described particularly in EPE [101]. In contrast to ferritin, erythroferrone levels are independent of inflammatory status in the woman, measured as levels of IL-6 [101], suggesting that the degree of inflammation does not affect erythroferrone levels.

Extracellular haemoglobin or free heme is highly toxic and pro-inflammatory, and causes oxidative stress by promoting the formation of reactive oxygen species (ROS), leading to lipid peroxidation, and effects DNA and protein damage [102, 103]. The human body has several endogenous defense proteins that attenuate the oxidative stress that is induced by free hemoglobin or free heme. Two of these defense proteins are discussed below.

Alpha-1-microglobulin

Alpha-1-microglobulin (A1M) (**Figure 6**) is a protein in the lipocalin family and has both general antioxidative properties and heme-binding capacity [104]. It is synthesized in the liver; distributed in the circulation bound to IgA (50 %), albumin (7%) and prothrombin (1%); and degraded by the kidneys [105, 106]. Most A1M is filtered in the primary urine, but a considerable amount is reabsorbed and catabolized in proximal tubular cells. Increased urine concentrations of A1M can thus be used as an indicator of tubular damage [107].

Alpha-1-microglobulin is up-regulated under conditions of increased oxidative stress, such as PE [108, 109]. Previous studies have found higher levels of A1M in PE and in women with a high risk of developing PE [109, 110]. This pattern has been interpreted as reflecting activation of endogenous defense mechanisms against oxidative stress.

Further, a recombinant version of A1M has been shown to ameliorate PE symptoms in animal models [111, 112]. Thus, A1M has a crucial function in mitigating the oxidative stress in PE, rendering it a potential therapeutic candidate for this syndrome.

Hemopexin

Hemopexin (**Figure 6**) is a glycoprotein that is synthesized primarily in the liver. Of all plasma proteins, it has the highest affinity for heme and it protects the cells against oxidative stress [113]. Like that of other acute phase reactants, the production of Hpx increases in inflammatory processes. Worse outcomes in sepsis correlate with lower levels of Hpx [114]. Hemopexin is also critical for activation of the enzyme heme oxygenase-1 (HO-1) [114], which is induced during oxidative stress and is the rate-limiting enzyme in heme degradation. Heme oxygenas-1 is downregulated, is less active, and correlates inversely with blood pressure in PE [115].

Hemopexin prevents heme-mediated oxidative stress by forming a complex with heme. This complex binds to the heme-Hpx receptor (LDL-related protein 1, or CD91), becomes internalized by macrophages through endocytosis, and is dissociated through lysosomal activity in which heme is further catabolized [116]. The Hpx-heme interaction is crucial for heme clearance [117].

Plasma Hpx rises in normal pregnancy. Lower plasma concentrations of Hpx in PE have been described as a sign of consumption due to oxidative stress [109, 115, 118].

Notably, Hpx downregulates angiotensin II receptor 1 expression and promotes vasodilatation. The decreased plasma Hpx concentration in PE may result in greater expression of angiotensin II receptor 1 and vasoconstriction [119, 120], perhaps explaining why levels of Hpx correlates with the blood pressure levels [108].

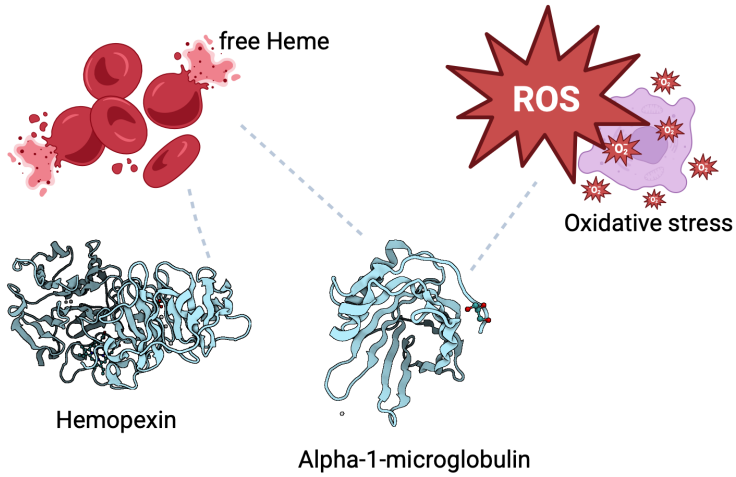

Figure 6
Hemopexin (Hpx) has an important role in binding free heme. Alpha-1-microglobulin is an antioxidant crucial for ameliorating the heme-induced oxidative stress in PE as well as oxidative stress arising from other sources. Figure created in Biorender.com.

Angiogenic imbalance

Angiogenesis is the formation of blood vessels. In PE, there is an imbalance between the proangiogenic biomarker PlGF and the antiangiogenic factor sFlt-1. Placental growth factor binds to vascular endothelial growth factor (VEGF) receptor 1, which promotes the binding of VEGF to VEGFR2, contributing to endothelial NO-production. Soluble fms-like tyrosine kinase-1 resembles VEGFR1 and binds to both PlGF and VEGF. The upregulation of sFlt-1 and the consequent decrease in PlGF mirrors the STB stress in PE when triggered by hypoxia [12, 121]. An imbalance between proangiogenic and antiangiogenic factors affects endothelial function – for example, by reducing NO [30] – and apheresis and removal of sFlt-1 reduce symptoms and prolong pregnancy in PE [122].

The sFlt-1:PlGF ratio is the first predictive biomarker for PE that has been introduced in clinical practice. This ratio rises several weeks before the debut of symptoms and may correlate to severity and adverse pregnancy outcomes [123]. Although this ratio is being continuously evaluated in studies for its generalisability in various clinical settings, it has not yet been implemented in Swedish maternal healthcare. The Fetal Medicine Foundation (FMF) model for predicting foremost EPE, incorporates a combination of maternal risk factors, MAP, uterine artery pulsatility index (PI) and PlGF [124]. This model is used to guide prophylactic treatment with aspirin, which, according to the ASPRE-trial, can reduce the rate of preterm PE by 62 % [125, 126].

Soluble fms-like tyrosine kinase-1 (sFlt-1)

As discussed, sFlt-1, is a soluble VEGF receptor and exerts its antiangiogenic effects by binding to PlGF and VEGF [127]. It antagonizes VEGF signalling, leading to reduced NO-synthesis and enhanced endothelin-1 generation and, consequently, vasoconstriction [128-131] (**Figure 7**). In PE, overexpression of sFlt-1 increases angiotensin II sensitivity, further contributing to an increased state of vasoconstriction despite lower circulating levels of angiotensin II [132].
Soluble fms-like tyrosine kinase-1 is released from STB-generated exosomes into the maternal circulation. It binds to heparan sulfate in the endothelial glycocalyx, damaging the glycocalyx, and thus increasing leukocyte adhesion to the endothelium and contributing to vascular inflammation and tissue injury [70, 133].

The ratio of sFlt-1 to PlGF

In normal pregnancy sFlt-1 begins to rise in gestational Week 30–32, whereas PlGF levels peak at approximately 30 gestational weeks and then decline in plasma [30]. Further, placental function decreases at the end of pregnancy, resulting in STB stress during the last 8–10 weeks of gestation [30].

The ratio between sFlt-1 and PlGF has been proposed as a potent predictor of the development of PE symptoms and adverse pregnancy outcomes. In women who are at less than 34 weeks of gestation, an sFlt-1:PIGF ratio ≥ 85 correlates with a diagnosis of PE and adverse pregnancy outcomes and delivery within 2 weeks [134-137]. Moreover, the sFlt-1:PlGF ratio has been shown to be a superior predictor of maternal adverse events compared with the full PE Integrated Estimate of Risk score (PIERS) [138].

An sFlt-1:PlGF ratio of ≥ 85 has a sensitivity of 88 % and a specificity of 99.5 % in identifying women with PE [136]. Further, a ratio of ≤ 38 has a negative predictive value of 99.3 % for excluding out PE within 1 week [136, 139]. Logically, the sFlt-1:PlGF ratio is more suitable for predicting and diagnosing EPE than LPE, given the angiogenic imbalance that arises already at the beginning of pregnancy in EPE due to an insufficient placental development, leading to STB stress. Conversely, in LPE, the placental insufficiency that increases this ratio does not become evident until the end of pregnancy. Also, for LPE, the diagnostic threshold has been raised to ≥ 110 [140] to compensate for the naturally decline in placental function at the end of pregnancy that affects the ratio.

Postpartum, sFlt-1 levels decreases more rapidly than those of PlGF. Soluble fms-like tyrosine kinase has been suggested to have a half-life of 1.4±0.3 days, versus 3.7±4.3 days for PlGF. Further, the concentration of PlGF rises in some patients postpartum before declining, indicating that PlGF also originates from non-placental sources [141]. These sources can be erythroblasts, and there has also been described and interplay between angiogenic factors, PE development and thyroidal function [142-144].

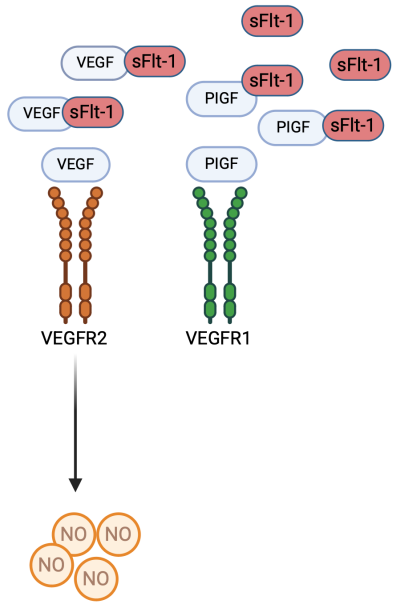

Figure 7
Placental growth factor (PlGF) binds to vascular endothelial growth factor receptor 1 (VEGFR1) which leads to a higher fraction of vascular endothelial growth factor (VEGF) binding of VEGFR2. This in turn, leads to increased levels of NO. Soluble fms-like tyrosinkinase -1 binds to both PlGF and VEGF, which will reduce NO-production by blocking VEGF from binding VEGFR2. Figure created in Biorender.com.

Sphingosine-1-phosphate

Sphingosine-1-phosphate (S1P) is a bioactive sphingolipid with several important biological functions, which depend on the plasma S1P concentration and type of receptor that is activated. Sphingosine-1-phosphate is produced by platelets, erythrocytes, leukocytes and endothelial cells through the phosphorylation of sphingosine, catalyzed by sphingosine kinase 1 and 2 (SphK1 and SphK2, respectively) [145] (**Figure 8**). This sphingolipid is metabolized primarily through enzymatic degradation and hepatic uptake rather than renal clearance and may thus be independent of kidney function [146]. In the blood stream, S1P is bound primarily to high density lipoprotein (HDL)(Apolipoprotein M) and to a lesser extent albumin (< 30-40 %) [146, 147]. Sphingosine-1-phosphate exerts its effects by acting on G-protein coupled receptors, S1P receptor 1-5 (S1PR 1-5), of which S1PR 1-3 are specific for the cardiovascular system, regulating vascular tone, endothelial permeability, and cardiac remodeling and fibrosis [147]. Moreover, S1PR1 is the predominant receptor that is expressed by the vascular endothelium, whereas S1PR2 and 3 are present at lower levels. Sphingosine-1-phosphate is crucial for maintaining the integrity of the endothelial barrier [148-150], and S1PR1

appears to be the most important receptor for barrier maintenance, reduced vascular inflammation, and induction of vasodilation via endothelial NOS [151]. Notably, S1PR2 levels are higher in serum and placental tissue of rats with induced PE [152]. Blocking S1PR2 reduces blood pressure and the levels of inflammatory mediators, such as the cytokines TNF-α, and IL-6 [152].

The influence of S1P on vascular tone depends on the activation status of its receptors present on the vascular endothelium or on vascular smooth muscle cells. Activation of S1PR1 and 3 on the vascular endothelium leads to vasodilatation via the endothelial NOS pathway, whereas activation of S1PR2 and 3 on vascular smooth muscle cells promotes vasoconstriction [153]. Sphingosine-1-phosphate has been linked to several inflammatory diseases and has been shown to have immunomodulatory properties [154, 155]. Vascular inflammation is an important component in the development of hypertension and CVD. Shingosine-1-phosphate may thus be a significant factor in the development of CVD – abnormal levels of S1P have been observed in patients with hypertension compared with healthy controls [156]. Further, S1P correlates with blood pressure and aortic stiffness in adolescents after preterm birth – patients who are at increased risk of developing CVD [157].

High-density lipoprotein levels increase in healthy pregnancy, and HDL-bound S1P induces vasodilatation via NO and protects against the vascular inflammation in arteriosclerosis [146]. However, S1P appears to harbor both proatherosclerotic and antiatherosclerotic properties, wherein HDL-bound S1P protects vessels, whereas albumin-bound S1P exhibits pro-arteriosclerotic properties [158]. Further, the increase in HDL is less prominent in PE, which may contribute to the endothelial dysfunction that occurs in this syndrome [146]. Also, the LDL:HDL ratio is higher in early pregnancy in women who later develop PE [159].

In contrast to sFlt-1, S1P is involved in the synthesis and stabilization of the endothelial glycocalyx by suppressing matrix metalloproteinases (MMPs) activity that would otherwise induce shedding [160-163].

Sphingosine-1-phosphate is involved in placental development and may have a role in the pathophysiology of PE by affecting trophoblast differentiation and syncytialization [164-168]. Signaling via S1PR1 appears to maintain adequate placental function, whereas S1PR2 is up-regulated in such pathological conditions as PE [167, 169]. Further, proinflammatory TNF-α -induced S1P signaling has been shown to lead to defective placental development [170]. Previous studies on S1P concentrations in plasma and placental tissue have generated conflicting results, reporting higher and lower plasma S1P concentrations in PE compared with healthy pregnancy [171-173]. However, the validity of these data is limited, given the small sample size and heterogenous nature of these studies with regard to their inclusion criteria.

Sepsis is an inflammatory disease that has common features with PE clinically, with similar organ involvement and dysfunction, and pathophysiologically, sharing mediators and signaling pathways. As in sepsis, vascular permeability increases in

PE, leading to fluid leakage and edema. Lower levels of S1P – specifically HDL-bound S1P – correlate with the severity of sepsis [67, 174]. Further, albumin substitution improves outcomes in sepsis, an effect that has been attributed to the dependence on albumin as a carrier of S1P [175].

It is unknown whether the albumin leakage that is often seen in PE due to endothelial glycocalyx and podocyte damage in the kidneys [176], has a clinically relevant impact on S1P levels.

Sphingosine-1-phosphate signaling pathways constitute an interesting potential target for the treatment of PE, as been discussed by Kerage et al., perhaps through administration of an S1PR1 agonist [177] or a compound that blocks S1PR2 [152].

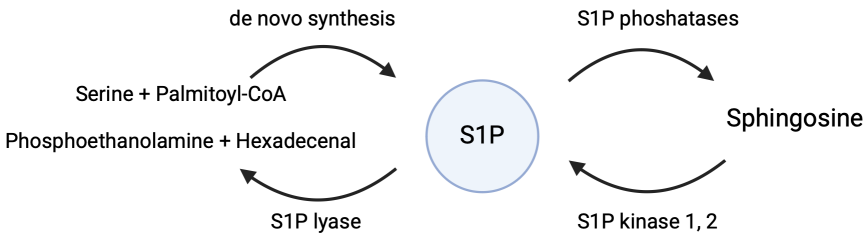

Figure 8
Metabolism of sphingosine-1-phosphate. Figure created in Biorender.com.

Machine learning models

Artificial intelligence (AI) is a collective term for several types of data science techniques, such as machine learning, deep learning, and artificial neural networks [178] (**Figure 9**) and is gaining increasing interest in general society and science. The outlook on AI harbors excitement regarding what the method can provide, but also with skepticism and sometimes fear over its potential to replace the human mind. Several medical specialities have been examining the use of AI for various purposes. For example, its excellent pattern recognition abilities render AI especially convenient for use in diagnostic radiology [179].

The discussion will hereafter focus on machine learning models, because that was the AI concept that was used in Paper II. The model development that was performed in this thesis will be expounded on in the methods section but is summarized briefly in **Figure 10**.

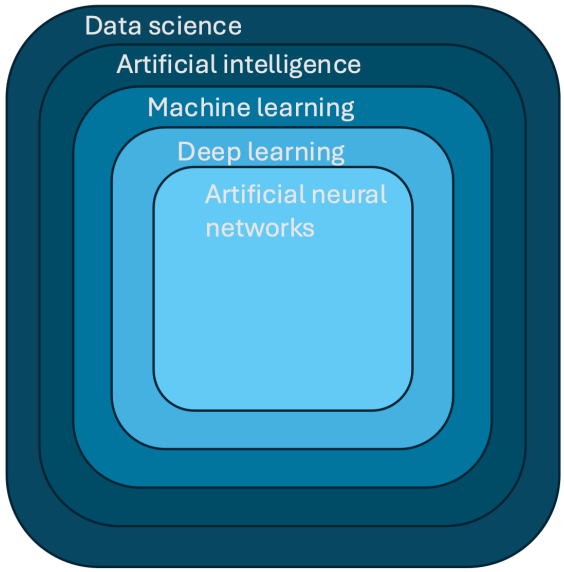

Figure 9
Various types of data science techniques of which machine learning models were used in Paper II. Figure inspired by Choi et al. 2020.

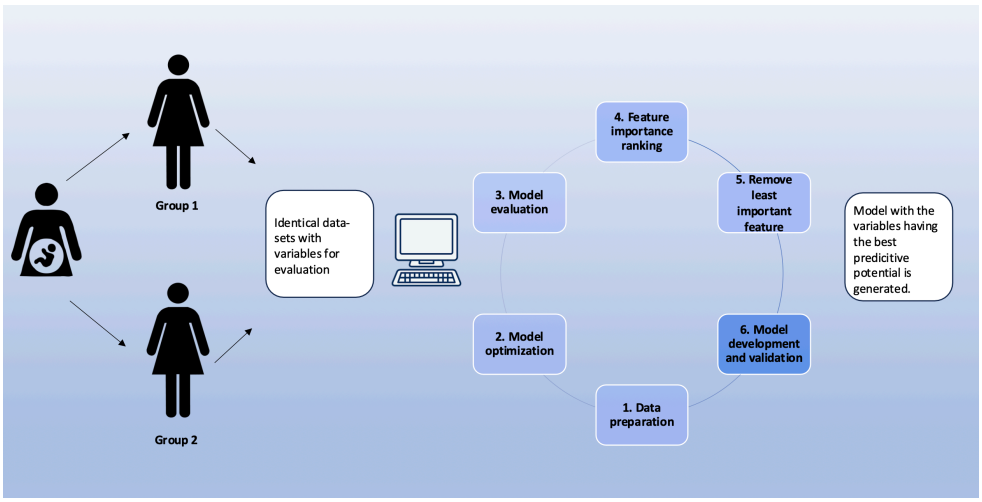

Figure 10
Schematic of the steps involved in the model development used in Paper II.

Prediction in the context of preeclampsia

An increasing number of publications are evaluating the predictive potential of machine learning models for the development of PE. However, several of them suffer from the absence of model validation, which renders them less generalizable. An optimal prediction model should include parameters that are easily obtainable even in a low-resource setting, in which the benefits are likely the largest. The ability to predict the development of PE may help obstetricians determine which patients to treat more expectantly, and whom to monitor more closely. Also, the ability to predict those patients who will experience severe pregnancy outcomes, may affect their treatment, monitoring intervals, and time of delivery.

With regard to predicting the development of PE, several machine learning models have been applied for model development, such as the gradient-boosted tree algorithm by Li et al. [180] and Maric et al. [181]. Li et al. included 38 clinical parameters that were collected from early second trimester records, of which the best predictors for the development of PE were fasting plasma glucose, mean blood pressure and BMI, with an area under the curve (AUC) of 0.955. Their model was internally and externally validated – but in the same population. Maric et al. reached an AUC of 0.89 with their model including 67 clinical parameters, of which chronic hypertension, parity, history of PE, type I diabetes mellitus, mean systolic and maximum diastolic blood pressures, elevated fasting glucose, and proteinuria had the best predictive potential. Also, height was inversely associated with PE.

As discussed, the development of EPE is often easier to predict, because this type of PE is an ongoing process, starting with defective placental development, whereas in LPE, placental development is normal. However, Jhee et al. [182] developed a prediction model for the occurrence of LPE after 34 weeks of gestation, based on data from the early second trimester until gestational Week 34. Systolic blood pressure, serum blood urea nitrogen, creatinine, platelet count, serum potassium levels, serum calcium levels and urinary protein were the most predictive of the development of LPE, with AUC values ranging from 0.776 to 0.924, depending on the model used. Several other parameters differed significantly between the groups, but did not turn out to be decisive for the final prediction model [182].

Not all studies have demonstrated the superior predictive potential of machine learning models. Sandström et al. [183] included several categorical variables, (i.e. risk factors for PE), and numerical variables such as blood pressure, in predicting the development of EPE and LPE. In this case, multivariate regression models exceeded the performance of a machine learning algorithm.

A recent systematic review on the use of machine learning models in predicting the development of PE comprised 4 studies, 3 of which have been discussed above (Li et al., Jhee et al. and Manic et al.). The review concludes that machine learning models show high predictive performance based on variables from routine prenatal care visits, such as maternal characteristics, medical history, medications, information from previous pregnancies, and laboratory and ultrasound findings [184].

Another aspect of prediction is the potential to foresee severe pregnancy outcomes in PE that has already manifested. Schmidt et al. [185] studied 114 features in a cohort of 1647 women, including sFlt-1:PlGF ratio and Doppler data (umbilical artery PI, middle cerebral artery PI, mean uterine artery PI). The model generated an AUC of 0.82 for predicting a severe fetal or maternal outcome in relation to PE at any time in the pregnancy until 2 weeks postpartum. The most important parameters were gestational age, sFlt-1:PlGF ratio and maternal height.

The Preeclampsia Integrated Estimate of Risk Score (PIERS) is the most widely validated risk score that is used in PE, having moderate accuracy [186]. When applying this model in an international context, combining it with machine learning, and including routine parameters that can be obtained in a clinical context from low-to-high income countries, the model`s predictive potential increased from an AUC value of 0.68 to 0.80 [187]. In grading the importance of features in the PIERS-ML model the highest-ranking variables were elevated serum creatinine, aspartate and alanine transaminases (ASAT and ALAT), and low values of platelet count, oxygen saturation, and hematocrit values. Also, a high national maternal mortality ratio and a low national per capita GDP were associated with adverse outcomes. However, when used for consecutive prediction, the model has been shown to have a worse predictive potential over time [188].

Rationale for the thesis

Many pathophysiological pathways that drive the development of PE have been described in previous research, but few studies have centered on the characteristics of severe PE with multiorgan failure and need for intensive care compared with less severe PE cases.
This thesis will focus on the clinical features of severe PE and how they are reflected as biomarkers for oxidative stress, angiogenic imbalance, endothelial damage, and vascular regulation (Papers I and III).

The ability to predict severe outcomes in PE, may optimize care and influence decision-making in obstetrics – for example, with regard to the timing of delivery. The use of machine learning in this area of medical research has generated promising results. Few published studies have applied machine learning for predicting adverse outcomes in manifest PE [185], including such parameters as biomarkers sFlt-1 and PlGF. These biomarkers, however, remain to be implemented in Swedish healthcare. This thesis will examine whether a prediction model for severe PE with need for intensive care can be developed using clinical routine parameters that are available in most health care settings in highly developed countries, such as Sweden (Paper II).

Preeclampsia is associated with an increased risk of developing chronic hypertension and other manifestations of CVD. Its etiological mechanisms are not entirely known, nor are the subtypes of PE that elevate the risk of CVD. In paper IV, cardiovascular status in previous severe PE cases, 4–7 years after delivery, was evaluated and compared with women with normotensive pregnancies during the same time period by measuring blood pressure and evaluating cardiac anatomy and function by cardiac magnetic resonance imaging (CMR) and echocardiography (Paper IV).

Aims of the thesis

The overall aims of this thesis were to describe various aspects of the pathophysiology of severe PE, determine whether deterioration of PE is possible to predict, and examine the relationship between severe PE and the potential increase in risk of future CVD.

The specific aims for each study, presented as Papers I-IV, are:

I. To evaluate clinical risk factors, and analyze the biomarkers Hpx, A1M, and the sFlt-1:PlGF ratio, in PE pregnancies that require intensive care, compared with uncomplicated PE cases and controls.

II. To develop a prediction model for severe PE with multiorgan failure and need for intensive care, based on clinical parameters and routine lab biomarkers, using machine learning models.

III. To assess the biomarker S1P as a marker of endothelial injury and disease severity in PE pregnancies that require intensive care, versus uncomplicated PE cases and controls.

IV. To evaluate long-term cardiovascular health in a cohort of women with severe PE that involved intensive care after delivery compared to normotensive pregnancies (the PRE-HEART study).

Methods

Ethical considerations and reflections

All studies were approved by the Swedish Ethical Review Authority (dnr. 2016/49, 2019-0468, 2021-06884-01, 2022-02271-02 and 2015/267 for SWECRIT) and performed per the ethical rules for medical research, as stated in the Declaration of Helsinki. All study participants were able to understand the given information and gave their written consent. In all studies, they were informed about the possibility of having their samples destroyed or discontinuing their participation at any point.

Biobank blood samples and routine laboratory parameters were measured in the PE cases. The blood samples from normotensive controls were collected only for the biobank study. Pregnant patients routinely have a venous line inserted during delivery for safety reasons; thus, venipuncture is an expected route for blood sampling during delivery that carries negligible harm for the patient. Due to the unstable condition of intensive care patients, an arterial cannula was also inserted into all patients in the ICU cohort for invasive blood pressure monitoring and blood sampling.

Placental tissue was collected after delivery (vaginal or by Cesarean-section), with no harm to the mother or newborn.

In the PRE-HEART study (Paper IV) all cardiac assessments were noninvasive and pain-free and none was associated with any radiation. No contrast solution was administered. The clinical research team was prepared to handle pathological findings that were not associated with the cardiovascular system, as well as eventual cardiovascular abnormalities. For Study IV, normotensive pregnancies were selected from previous CMR imaging studies as controls, to be able to compare cardiovascular status and eventual secondary findings with previously acquired images: this aspect will be addressed in “Study population”. All included women were encouraged to contact the research group with any general questions or specific concerns about the study results.

Study population

The intensive care cohort (**Figure 11**) included in Papers I–IV originated from the SWECRIT study (Blood Samples from Critically Ill Patients and Healthy controls, ClinicalTrials.gov ID NCT04974775). This project collected blood samples from all intensive care patients in Skåne County, Sweden from 2015-2018. All samples were drawn within 1–2 hours of admission. The purpose of this regional biobank was to enable future biomarker analyses in projects that are related to critical illness and intensive care. A total of 53 patients were identified as having been diagnosed with severe PE (early-onset and late-onset), eclampsia, and/or HELLP during this period in the PASIVA-register. Three patients with an incorrect diagnosis (based on their medical records), and 9 patients who lacked blood samples were excluded, yielding a total of 41 women who were eligible for inclusion. The time between delivery and blood sampling for biomarker analysis was 27 hours for all included cases. All samples were taken postpartum. No placental tissue was collected from this group. Plasma and serum were collected at these samplings and stored at -80 ^{o}C.

A subgroup of 10 women from the intensive care cohort were included in Paper IV: the PRE-HEART-study. These women were chosen, based on severity, defined as severely dysregulated blood pressure, HELLP or eclampsia as indications for admission to the ICU. By definition, eclampsia and HELLP are severe forms of PE.

The control cases in Papers I–III (**Figure 11**) comprised blood samples that were collected prepartum and postpartum from PE patients (EPE and LPE, severe and mild, not admitted to intensive care) and normotensive pregnancies at Skåne County University Hospital in Malmö and Lund, Sweden. These samples were stored in a biobank in the Department of Obstetrics and Gynecology, Lund University, Sweden. Postpartum placental tissue samples were also collected for these 2 study groups.

The primary outcome in Papers I–III was multiorgan failure with need for intensive care. Considering the heterogeneity of PE, no other discriminator between groups was chosen to avoid selection bias.

The aim of Paper II was to develop a prediction model for intensive care needs in PE; thus, normotensive controls were not included in this study.

The exclusion criteria for Paper IV were previous CVD, pulmonary disease, smoking within 10 years, diabetes mellitus (gestational or pregestational) and contraindications for CMR. The controls were matched to the women with prior PE for level of physical activity. In Paper IV, 10 previous normotensive pregnancies were included as controls;.these women had participated in CMR studies that were related to PE as normotensive controls during their index pregnancy. This new control group was introduced foremost to be able to use previously acquired CMR images for comparisons in case of unanticipated pathological findings.

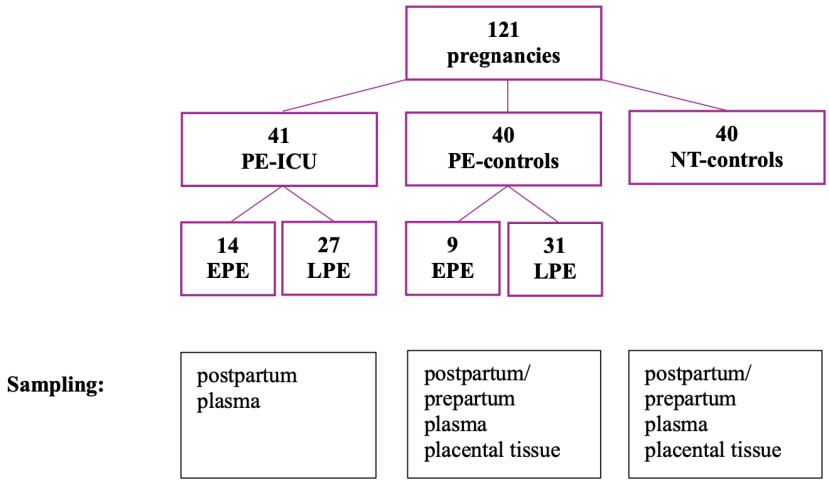

Figure 11
Flow-chart of included pregnancies and types of sampling for biomarker analysis in Papers I and III. This figure was previously published in Paper III, Edvinsson et al. [189]. ICU – intensive care unit, EPE - early-onset PE, LPE - late-onset PE, NT – normotensive.

Analytical methods

Enzyme-Linked Immunosorbent Assay (ELISA)

Enzyme-Linked Immunosorbent Assay (ELISA) was used in Papers I and III to determine the blood plasma concentrations of A1M, Hpx, HA, SDC-1 and HPSG2.

Enzyme-Linked Immunosorbent Assay is a common method for measuring proteins and glycoproteins. All ELISAs in this thesis – except that for A1M, which was an in-house A1M-specific ELISA – were performed by using commercial kits. The advantages of commercial kits, in addition to their practicality, are that they have already undergone quality controls and have predetermined intra-assay and inter-assay variabilities.

Sandwich ELISA was performed in this thesis (**Figure 12**), in which 2–3 sets of specific antibodies are used to detect the antigen of interest. With commercial kits, the wells of the plates are already pre-coated with an immobilized antigen-specific capture antibody, whereas in our in-house ELISA, the first step is to coat the ELISA-plate overnight with the capture antibody. Samples and standards are then added to duplicate wells, and antigen that is present in the samples will bind to the immobilized capture antibody. The "sandwich" is formed through the addition of a second antigen-specific detector antibody that is labeled with biotin or an enzyme, such as horse radish peroxidase (HRP). If a biotin-labeled detector-antibody is used,

streptavidin-labelled horseradish peroxidase solution is added in the next step. In the final step, a substrate solution is added, reacting with the enzyme-antibody-target complex to generate a measurable signal that is detected on a microplate reader. The intensity of this signal is proportional to the concentration of antigen in the original sample. Determination of the antigen concentration in a sample requires the construction of a standard curve, which is based on known concentrations of the antigen in standard samples (**Figure 13**).

All commercial ELISA kits were used according to the manufacturers` protocols. The ELISA for A1M will be detailed in the next section.

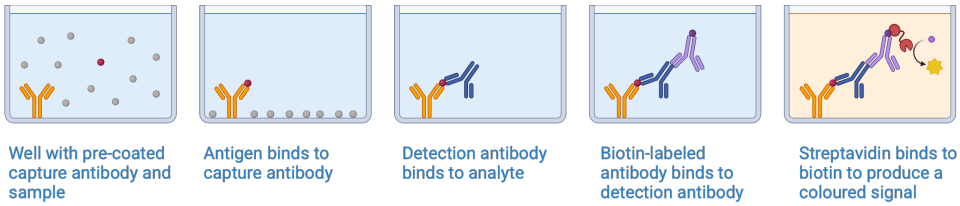

Figure 12
Steps of a sandwich ELISA. This particular ELISA was used for HSPG2, involving a third antibody, because the “detector-antibody” in the illustration lacks biotin, which is conjugated to another antibody that is added in Step 4. In Step 5 a subtrate solution is added to produce the coloured signal. Biotin increases the sensitivity by amplifying the signal. Figure created in Biorender.com.

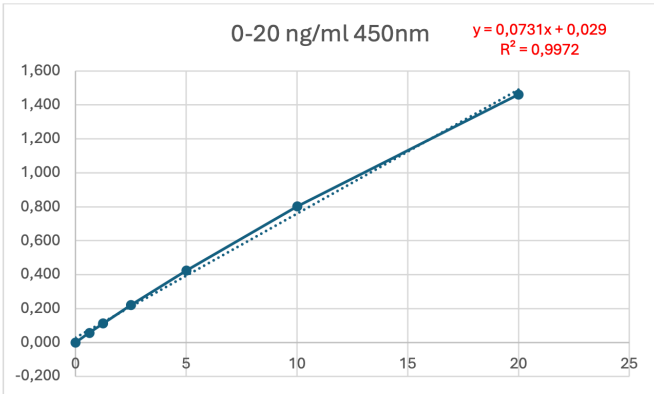

Figure 13
Example of a standard curve, here represented by the analysis of HA in the ICU-cohort. Concentration expressed in ng/ml is shown on the x-axis and absorbance is shown on the y-axis. An R^2 value near 1 (>0.95) indicates a good correlation and a trustworthy analysis.

Alpha-1-microglobulin ELISA

A sandwich ELISA that was developed in-house was used to quantify A1M, as described by Youssef et al [109]. Microtiter plates were coated with an anti-human A1M antibody (mouse monoclonal, clone 35.14; 5 µg/mL in phosphate-buffered saline (PBS) overnight at 4°C under sealing film with 100 mL/well. After the wells were washed 3 times with PBS + 0.05% Tween-20, 100 mL of human urinary A1M standard samples (1.56–100 ng/mL in PBS + 0.05% Tween-20) and diluted blood plasma samples were added to the wells and incubated under sealing film for 1 h at room temperature, in the dark with rotational shaking at 250–500 rpm. After the wells were washed 3 times with PBS + 0.05% Tween-20, 100 mL/well of a horseradish peroxidase (HRP)-conjugated detection antibody (mouse monoclonal, clone 57.10; 5 ng/mL) was added and incubated under sealing film for 1 hour at room temperature, in the dark with rotational shaking at 250–500 rpm. Finally, after 3 washes with PBS + 0.05% Tween-20, 100 mL/well of a ready-to-use 3,3´,5,5´-Tetramethylbenzidine (TMB, Life Technologies, Stockholm, Sweden) substrate solution was added under sealing film and incubated without shaking. The reaction was stopped after 20 minutes with 1 M sulfuric acid and the absorbance was read at 450 nm on a GloMax microplate reader. A standard curve based on the human urinary A1M standard samples was used to measure the concentration of A1M in each sample. This in-house assay has reported intra-assay coefficient of 2.23 % [109].

Mass spectrometry

Lipid extraction for S1P mass spectrometry was used in Paper III. Plasma samples were prepared as described by Chipeaux et al [190]. Briefly, 10 µL of plasma was mixed with 90 µL ice-cold methanol (MeOH) that contained 22 nmol/L S1P-D7 (Avanti Polar Lipids/Merck, Darmstadt, Germany) as an internal standard (ISTD) in 1.5 mL tubes and placed on ice. The tubes were vortexed vigorously for 10 seconds, which was repeated after 15 min on wet-ice. After a 30-min incubation on ice, the samples were centrifuged at 20.000 g for 10 min at 4°C. The supernatant was transferred to an autosampler vial and stored at -80°C until mass spectrometric analysis.

Sphingosine-1-phosphate was extracted from placental tissue using an adapted protocol originally described by Bode and Gräler [191]. Briefly, placental tissue samples were weighed (in mg), mixed with 1 mL PBS and homogenized using an Ultra-Turrax® homogenizer. Homogenate volumes that were equivalent to 20 mg of tissue were added to a glass extraction vial, and PBS was added to a total volume of 1 mL. Fifty pmol of ISTD S1P-D7 (5µM, Avanti Polar Lipids/Merck, Darmstadt, Germany) was added, and the suspension was mixed with 1 mL MeOH, 200 µL 6 M hydrochloric acid and 2 ml chloroform. After 3 min of vigorous vortexing, the samples were centrifugated at 1 900 g for 3 minutes. The lower organic phase was

transferred to a clean glass tube using a glass Pasteur pipette. The previous steps, including the addition of chloroform and centrifugation, were repeated with the remaining aqueous phase, and the 2 organic phases were then combined, and chloroform was evaporated under a nitrogen stream in a fume hood. Dried lipids were dissolved in 100–200 µL MeOH and transferred to an autosampler vial. A 6-point standard curve was generated from extracts of 5–80 pmol S1P in fatty-acid free BSA/PBS applying the same extraction procedure.

The samples were analyzed by liquid chromatography-coupled tandem mass spectrometry on a 6495 QQQ instrument (Agilent Technologies, Sweden) with standard reference samples included. Briefly, the samples were separated by liquid chromatography and ionized. In the mass spectrometer the ions were charged by an electromagnetic field and released into a vacuum, wherein the kinetic energy of the charged ions was proportional to their mass. A detector registered the mass-to-charge ratio (m/z). According to Chipeaux et al. S1P has an m/z ratio of 380.2 > 264.2 [190] (**Figure 14**).

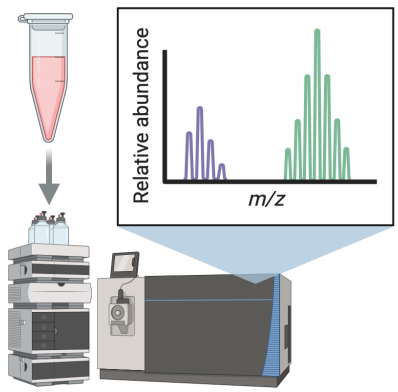

Figure 14
Liquid chromography-coupled tandem mass spectrometry instrument, with a diagram of the results of the sample analysis. The x-axis is labeled with m/z ratio which can often be approximated as the mass of the measured chemical sample. The relative abundance on the y-axis represents the signal intensity. Figure created in Biorender.com.

Imaging

The 20 women in the PRE-HEART study were examined by CMR and echocardiography, in addition to blood pressure measurements. Both CMR and echocardiography are non-invasive examinations and lack ionising radiation. Cardiac magnetic resonance imaging is a method for imaging that uses a magnetic field and radiofrequency waves to create images of the heart, for example. In the

MR imaging process, hydrogen atoms are used to generate signals and images, by applying a strong magnetic field, inducing hydrogen protons to align along the same longitudinal axis. Radiofrequency waves displace the alignment briefly, and when the waves are switched off, a signal is generated. Various coils can be used to strengthen the magnetic field around the part of the body that is examined.

Echocardiography entails the use of ultrasonography to image the heart. Ultrasound waves are generated by a piezo-electric crystal in a transducer. The ultrasound waves reflect when they meet various tissue boundaries and when the echoes bounce back at the transducer again, electric signals are generated, creating an image.

Cardiac magnetic resonance imaging (CMR)

Participants were imaged in the supine position using a 1.5 Tesla (T) CMR scanner (Aera, Siemens Healthineers, Erlangen, Germany) (**Figure 15**) with a combination of a spine coil and a cardiac array coil with retrospective electrocardiogram gating. Balanced steady-state free precession cine images were acquired at end-expiration in the standard short-axis and 4-, 3-, and 2-chamber views for measurements of volumes and function. For thoracic aortic pulse wave velocity (PWV), 2D phase-contrast flow with more than 35 timeframes over the cardiac cycle was acquired in free breathing in the ascending aorta and in the descending aorta at the level of the diaphragm, together with a white-blood 3D isotropic T2-prepared sequence for centerline distance.

Images were analyzed in Segment 4.0 R13109 (Medviso, Lund, Sweden) [192], with the observers blinded to all other data. Left endocardial and epicardial borders, and right endocardial borders were delineated manually in short-axis images at end-diastole and end-systole for ventricular volumes and LVM [193]. Long-axis images were used for guidance. Stroke volume was calculated as end-diastolic volume minus end-systolic volume (SV = EDV - ESV), and ejection fraction was calculated as SV divided by end-diastolic volume and expressed as a percentage (EF = (SV/EDV) x 100). Left ventricular mass was calculated as left ventricular wall volume multiplied by myocardial density (1.05 g/ml). To account for variations in body size, all variables except for ejection fraction were indexed to body surface area.

For calculations of PWV, aortic centerline distance was measured manually in the 3D white-blood images and defined as the distance between the flow planes in the ascending aorta and at the level of the diaphragm. The flow curves were obtained by automatic vessel delineation [194], with manual correction if needed. Pulse wave velocity was assessed using the time-to-foot approach, including baseline correction [195, 196].

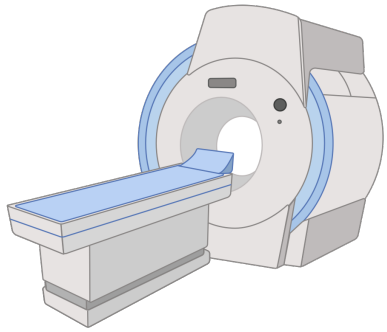

Figure 15
Magnetic resonance imaging machine. In the PRE-HEART study, the participants were imaged in a CMR scanner using a 1.5 T (tesla) magnetic field. Figure created in Biorender.com.

Echocardiography

Echocardiography was performed using a Vivid E95 ultrasound system (GE Ultrasound, Horten, Norway) according per current recommendations [197, 198] including an evaluation of diastolic dysfunction [199]. Images were stored and analyzed offline in EchoPac 11.0.0.0 (GE Ultrasound, Waukesha, Wisconsin). To reduce potential bias, all analyses were conducted by the same experienced sonographer in a blinded, randomized order after the completion of data acquisition. Mitral inflow was measured by pulsed-wave Doppler in the apical 4-chamber view. Trans-mitral early (E) and late (A) diastolic velocities were obtained, and the mitral E:A ratio was calculated. Mitral deceleration time was calculated as the time from maximum flow velocity over the valve until zero velocity.

Early mitral annular velocity (e´) was measured at the septal and lateral walls by Doppler tissue imaging, and the mean E:e´ ratio was determined. Global longitudinal strain was assessed by 2-dimensional speckle tracking and calculated as the mean of the global peak systolic strain from each of the 3 apical 2-, 3- and 4 chamber views. Anatomical view of the heart is shown in **Figure 16**.

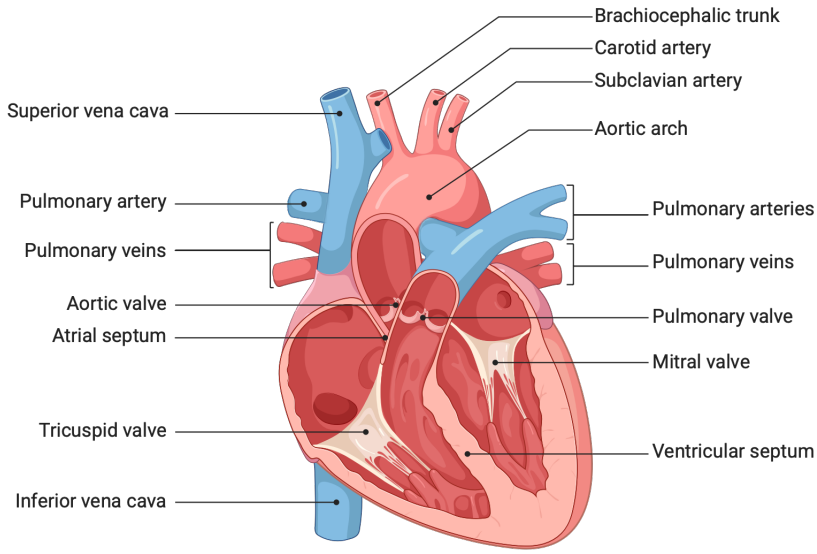

Figure 16
Anatomical 4-chamber illustration of the heart. Template from Biorender.com

Machine learning

The aim of Paper II was to develop at prediction model for intensive care need in PE, based on clinical routine parameters [200].

Due to a relatively small dataset, categorical variables were excluded and the numerical variables age, BMI, systolic blood pressure, diastolic blood pressure, platelets, ALAT, ASAT, creatinine, uric acid and hemoglobin values were included. To ensure an unbiased model development and independent internal validation, the data were split randomly into a training set (80 %) and a test set (20 %) in a stratified manner. Stratification ensured that the distribution of an outcome variable was approximately equal in the training and test sets, thus avoiding outliers or discrepant values in only 1 of the groups. Thus, the patients in the training set differed from those in the test set.

The model development was performed trough an iterative (repeated) process to identify the subset of features that effected the best predictive performance. At the end of each iteration, the best model was used to determine feature importance, and the variable that was determined to be least important was removed. The iterative model development was completed when all, but 1 feature had been removed. The test set was then subjected to internal validation.

The tools and algorithms that were used in the model development are discussed below.

Shapley Additive Explanations (SHAP)

Variable or feature importance was calculated using Shapley Additive Explanations (SHAP) [201] – a method that explains the prediction or output from a machine learning model, based on the input. It calculates the contribution of individual features to the prediction in a given instance and answers the question of how the inclusion of a variable changes the model`s performance compared with when it is excluded. It is thus a useful tool to rank the importance of variables. Shapley Additive Explanations is helpful for understanding the often complex prediction model that is generated and can discover incorrect predictions. Also, SHAP can explain why a model works well on a dataset but performs worse on another. Using SHAP increases the trust of a model's predictive performance, and it is implemented easily in any other machine-learning model.

Optuna

Hyperparameters are configuration settings – i.e., variables that are set manually prior to the training of a model and that influences its learning process. They control such factors as model complexity and learning speed, but they are not learned from the data. The hyperparameters were tuned in an automated manner using Optuna, an open-source hyperparameter optimization framework for automating hyperparameter searches [202]. Optuna starts by randomly selecting a set of hyperparameters and trains the model. It evaluates the performance of the model and adjusts the hyperparameters to improve it. Optuna can be applied in deep learning, logistic regression, and decision trees, and uses the Tree-Structured Parzen estimator (TPE), explained below.
In summary, Optuna optimizes the hyperparameters to generate the most accurate model as efficiently as possible.

Tree-Structured Parzen estimator (TPE)

Tree Structure Parzen estimator (TPE) is a Bayesian optimization technique that intelligently searches for optimal hyperparameters. It separates the hyperparameters into those with high accuracy that performed well, and those with lower accuracy that performed poorly. This means that the model avoids wasting time on random choices.

Extreme Gradient Boosting (XGBoost)

EXtreme Gradient Boosting (XGBoost) is a machine learning algorithm that has increased in popularity in data science due to its versatility and performance. The core idea behind XGBoost is boosting, an ensemble learning technique that works by building multiple small models – in this case, decision trees – and combining

them to make better predictions. Each tree in XGBoost is designed to fix the errors of the previous trees, allowing the algorithm to improve iteratively. Initially XGBoost makes a simple prediction – often a constant value, like the average of a target variable in regression tasks. Then, it calculates the extent to which these predictions deviate from the actual values: the errors or residuals. New decision trees are built to predict these residuals, focusing on reducing them stepwise. The final prediction is made by combining the outputs of all the trees, typically through addition. The XGBoost model is designed to prevent overfitting, and the few missing values present in the data are handled internally by the XGboost model.

Early stopping

Early stopping is a technique that is used during the training of a machine learning model to prevent overfitting. In early stopping, the performance of the model on the validation set is monitored during training, and the training is halted when the performance starts to decrease.

K-fold cross-validation

In K-fold cross-validation the training data are divided into K non-overlapping partitions or folds. The model is then trained on K-1 of the folds, and the remaining fold is used as the validation set. This process is repeated for a total of K times and ends when all folds have been used as the validation set once. This process is illustrated in the figure below **Figure 17**.

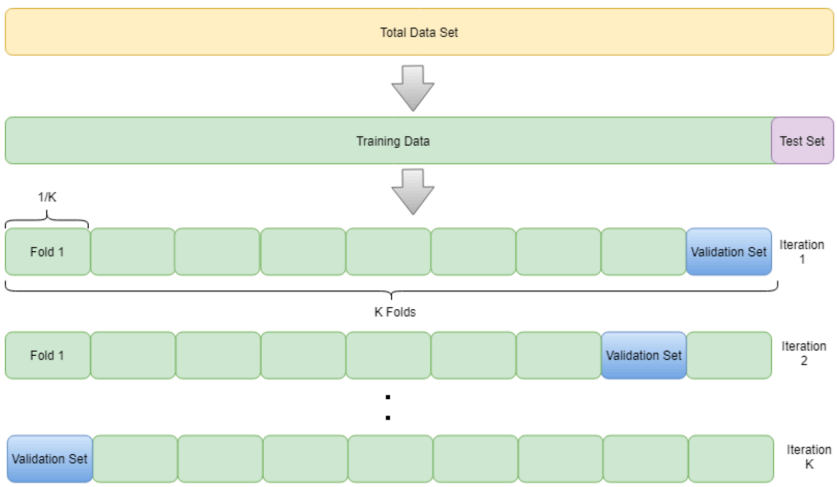

Figure 17
K-fold cross-validation. Illustration previously published in the supplement to Paper II, Edvinsson et al. [200].

Statistics

Statistical analyses were performed in R (version 4.2.2, R Foundation for Statistical Computing, Vienna, Austria), SPSS (version 30, IBM, Armonk, NY, USA) and in Prism (version 10.2.0.335, GraphPad Software, La Jolla, CA, USA).

No power calculations were made in Papers I and II, but the number of controls was matched to the number of patients in the ICU cohort. However, a power calculation was performed in Paper III to ensure that the number of participants was sufficient, based on previous studies on blood pressure measurement and vascular function, to estimate at detectable difference. A minimum of 32 samples per group was calculated to be required, using human reference values for plasma S1P concentration (757.4 nmol/L) and assuming a 12% change in plasma S1P in patients with PE.

For Paper IV the number of participants was based on sample size calculations in Bellenger et al. [203], considering the high precision of CMR, combined with experience from previous CMR measurements after pregnancy that is complicated by PE. Interobserver agreement was calculated by Bland-Altman method [204] and presented as bias (95 % limits of agreement).

In general, all results were expressed as mean (95 % confidence interval (CI)), mean (± standard deviation (SD)), median (interquartile range), or percentage as appropriate. Normality was assessed by histogram, Shapiro-Wilks test, and measurement of skewness for the data.

Differences between groups were assessed by Kruskal-Wallis test or one-way-ANOVA test for overall p-values, followed by Mann-Whitney unpaired test or t-test, depending on the distribution of the data. Categorical data were compared by Fisher`s exact test. P values ≤ 0.05 were considered to be significant. To evaluate the influence of confounding factors, the comparisons in Paper I were adjusted for differences in age > 40 years, BMI >30, duplex/multiple gestation, parity, previous PE or gestational hypertension, male fetal sex, gestational length, hypothyroidism and bleeding >500 mL at the time of delivery. In Paper III, regression-analyses were performed to assess the effect of confounding factors and changes throughout pregnancy, such as gestational age, blood loss at delivery, and kidney function.

Correlation analyses were performed using Spearman correlation coefficients and exact p-values were computed prior to correction for multiple comparisons by Bonferroni method.

Methodological considerations

Paper I

The Roche Elecsys assay® for measuring sFlt-1 and PlGF was available only for the analysis of serum samples – i.e., this analysis was only possible in the ICU-cohort and not in the PE or normotensive controls. The Thermofisher assay for plasma required 500 microliters of blood, which exceeded the total blood volume for each sample in the SWECRIT biobank. The study would have been strengthened by the ability to compare the sFlt-1:PlGF ratio for all study groups. However, because several previous studies have discussed the sFlt-1:PlGF ratio as a predictor of adverse pregnancy outcomes, additional information was gleaned in our study by measuring this ratio in the ICU-cohort, especially because the blood samples were taken postpartum, when the patients remained in an unstable clinical situation.

Because the ELISA for A1M was an in-house ELISA with a reported intra-assay coefficient of up to 2.23 % [109] this coefficient was calculated for all groups in Paper I to ensure accuracy. The resulting mean intra-assay coefficient was found to be lower in the current study, with a value of 1.3 %.

Paper II

The definitions of severe and other subtypes of PE are unfortunately insufficient to foresee which patients that will develop critical disease, necessitating the development of a prediction model for use in the clinic. One could consider the choice of the outcome measure in Paper II – that is, if more specific organ involvement would have added additional information to the prediction. However, because PE is a very heterogeneous disease, the choice of ICU care as our main outcome, contributes to the avoidance of selection bias, and distinguishes between severely ill patients who need continuous monitoring and less ill patients who can be managed in the delivery ward.

The development of the machine learning model can be complex to understand. It is thus important to note that several precautions have been taken to avoid overfitting in this relatively small dataset. This was achieved by using the XGBoost model, reducing the number of variables that was included, and assessing the model´s generalizability by cross-validation and internal validation on a test-set.

Paper III

The peer-review of Paper III raised the issue of how storage of the samples affected the stability of the analytes. Thus, additional statistical analysis was performed per Enroth et al. [205] to confirm consistency across collection periods for S1P, HA, SDC-1 and HSPG2 (2015-2018). By univariate linear modelling, sample age did

not affect analyte levels, based on storage time. Further, S1P levels in younger samples were lower than reference values for healthy individuals, indicating that S1P concentrations are not influenced by storage time [156, 174, 206]. Quality control samples for S1P harbored stable levels over a 3-year period. Stability also included the fact that some samples underwent several freeze-thaw cycles.

To ensure the stability and integrity of the analytes, the samples were processed and stored following strict protocols. Immediately on collection, plasma and tissue samples were stored at -80°C, minimizing the risks of degradation during processing. Samples from all groups underwent the same number of freeze-thaw cycles at the time of the individual experiments.

All ELISAs in this study were commercial kits, the mean intra-assay and inter-assay coefficients of variation (CVs) of which are as follows: Hyaluron Quantikine™ ELISA (Biotechne, R&D Systems): intra-assay CV <7.2% (n=20) and inter-assay CV<4.8% (n=20); Human Syndecan-1 ELISA (ThermoFisher Scientific): intra-assay CV <12% and inter-assay CV <10%; HSPG2 Human ELISA (Aviva Systems Biology): intra-assay CV <4.1% (n=20) and inter-assay CV <7.9% (n=20). Routine testing for the stability of glycocalyx degradation products using the commercially available ELISA kits was not performed. However, during pre-experimental testing of assay-specific sample dilutions, the levels of these products were stable over at least 1 freeze-thaw cycle (**Figure 18**). Also, the manufacturer`s recommendation to freeze the standard samples between experiments supports the stability of the assay under freeze-thaw conditions.

In evaluating the correlations, Bonferroni correction was used to adjust for multiple comparisons. This test, however, can be overly conservative and thus overlook significant correlations. In the absence of Bonferroni correction, more significant correlations were observed, but the stricter approach – applying this adjustment – was chosen in Paper III.

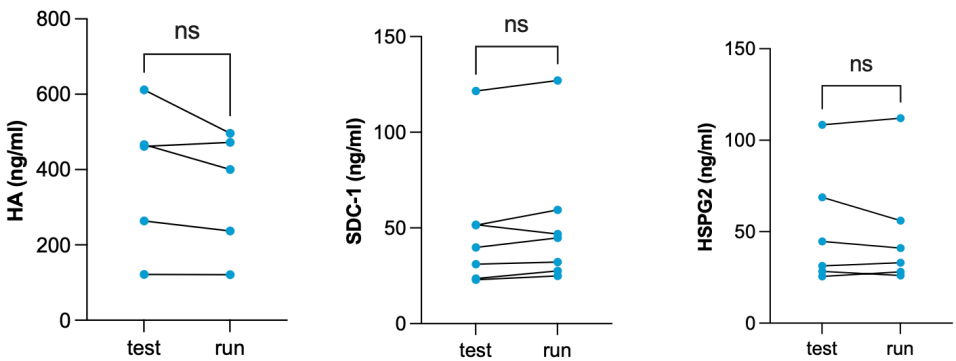

Figure 18
Stability of glycocalyx degradation products over 1 freeze-thaw cycle, as analyzed by commercial ELISAs.

Paper IV

The patient cohorts were dimensioned for CMR – not echocardiography; thus, statistically significant differences in ultrasound parameters could have been missed due to a small sample size (i.e. type II error).

If intravenous gadolinium contrast solution had been used, the myocardial tissue could also have been evaluated. As discussed, PE is associated with pathological cardiac remodeling. Signs of cardiac fibrosis could have been discovered if intravenous contrast solution had been used. However, this invasive procedure was not performed.

Participants self-graded their physical activity status, potentially introducing subjectivity and thus lack of trustworthiness of the data.

Results

Papers I and III

Characteristics of the study populations

Nine patients among the PE controls (22.5%) and 14 patients in the ICU cohort (34%) had EPE, whereas the remainder of patients in these groups had LPE.

The ICU cohort had more clinical risk factors for PE; was significantly older and had higher rates of *in-vitro* fertilization by egg donation, duplex/multiple pregnancies, and hypothyroidism. There was also an insignificant trend toward a greater proportion of male fetuses among the more severe PE cases. All PE cases had a higher BMI compared with normotensive controls. Intensive care patients had a significantly higher systolic and diastolic blood pressure despite being treated with several antihypertensive drugs versus to the other groups. The ICU patients also had significantly higher ASAT, ALAT, creatinine, and uric acid levels than PE controls. The clinical characteristics for the study groups are summarized in **Table 1.**

The reasons for admission to the ICU are listed in **Table 2**. The average ICU stay was 1.5 days. No patient who was cared for in the ICU for eclampsia was given prophylactic $MgSO_4$. Treatment with $MgSO_4$ was instead initiated in the ICU-departments.

Table 1
Clinical characteristics of the three study groups.

Clinical characteristics	ICU-cohort n=41	PE controls n=40	Controls n=40	p-value
Age (years)	33 (31–35) *	29 (28–31)	29 (28–31)	0.008
Parity (n)	0 (0–1)	0 (0–1)	0 (0–0)	0.030
BMI (kg/m^2)	27 (25–29)	27 (25–29) †	24 (23–26)	0.030
Gestation days at delivery (days)	250 (225–267) *	273 (256–278) †	282 (269–288)	<0.001
Highest systolic BP (mmHg) ‡	171 (164–178) *	160 (155–165) †	123 (121–126)	<0.001
Highest diastolic BP (mmHg) §	107 (103–112) *	101 (98–103) †	76 (74–70)	<0.001
Antihypertensive treatment (number of drugs)	0 = n 7 1 = n 9 2 = n 15 3 = n 10 *	0 = n 26 1 = n 13 3 = n 1 †	0 = n 40	<0.001
EPE/LPE (n)	14/27	9/31	0	-
Birthweight (g)	2155 (1551–3048) *	3131 (2346–3709)	3395 (3163–3775)	<0.0001
Gender (n) (%) Male Female Female & Male	 24 (58.5) 15 (36.6) 2 (4.9)	 20 (50.0) 18 (45.0) 2 (5.0)	 15 (37.5) 25 (62.5) 0	0.110

Values are shown as mean (95 % CI) for normally distributed data and median (quartiles) for non-normally distributed data. Exact p-values are given for Kruskal-Wallis test.

* Significant differences between ICU-cohort and PE controls by Mann-Whitney or unpaired t-test.

† Significant differences between PE controls and controls by Mann-Whitney or unpaired t-test.

‡ Highest systolic blood pressure recorded during hospitalization or in maternity care (1–30 days prior to delivery).

§ Highest diastolic blood pressure recorded during hospitalization or in maternity care (1–30 days prior to delivery).

Table 2
Reasons for admission to intensive care. Edvinsson et al. [200].

Reasons for admission to intensive care beyond PE	Number of patients
Dysregulated hypertension	n = 5
HELLP syndrome	n = 13
Postpartum hemorrhage	n = 8
Eclampsia	n = 9
Infection	n = 1
Kidney failure	n = 3
Pulmonary edema/heart failure	n = 2

Biomarkers

Hemopexin is lower in severe disease

The mean plasma Hpx concentration was 965±278 µg/mL in the ICU cohort, 1407±550 µg/mL in PE controls, and 1561±300 µg/mL in normotensive controls (**Figure 19**). Plasma Hpx concentrations were significantly lower in the ICU cohort compared with PE controls, with and without adjustments for potential confounders; kidney function (PE controls and ICU cohort) and bleeding at delivery. However, the difference between PE controls and normotensive controls was not significant. A subgroup analysis did not show any significant differences between EPE and LPE patients or between those with and without HELLP syndrome.

Alpha-1-microglobulin is lower in severe disease and in normal pregnancy

The mean plasma A1M concentration was 22.31±7.32 µg/mL in the ICU cohort, 25.92 ±8.0 µg/mL in PE controls and 20.65±6.47 µg/mL in normotensive controls (**Figure 19).** Normotensive pregnancies and the ICU cohort had significantly lower values compared with PE controls with and without adjustments for potential confounders; kidney function (PE controls and ICU cohort) and bleeding at delivery. In a subgroup analysis, there was no significant differences between EPE and LPE patients or between those with and without HELLP syndrome.

Postpartum ratio of sFlt:PlGF remained raised above the cut-off >85 (>110)

The median sFlt-1:PlGF ratio among all ICU patients was 293.8 (149.4-456) (**Figure 19**), broken down as follows: 263 (79,25–375,25) in EPE patients and 359 (166–507) in those with LPE. There was no significant difference between the 2 groups of ICU patients (p =0.08). Further, there was no correlation between this ratio and the plasma concentrations of Hpx, or A1M.

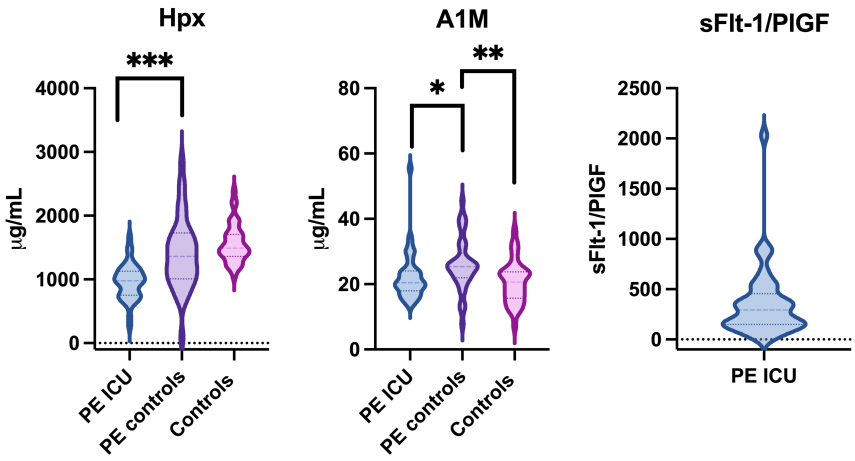

Figure 19
Distribution of hemopexin (Hpx) and alpha-1-microglobulin (A1M) in all groups. Distribution sFlt-1:PlGF ratios in all ICU-patients. * p ≤0.05, ** p ≤0.01, *** p ≤0.001, **** p ≤0.0001.

Sphingosine-1-phosphate is lower in severe disease

Postpartum plasma concentrations of S1P were lower in the ICU cohort (458 (399-595) nmol/L) compared to PE controls (678 (559-785) nmol/L) and normotensive controls (712 (572-901) nmol/L) (**Figure 20A**). This difference remained significant after adjustments for gestational age, kidney function (PE controls and ICU cohort) and amount of bleeding at delivery. There was no significant difference between PE controls and normotensive controls. In a subgroup analysis, there were no significant differences between EPE and LPE (**Figure 20B**).

Plasma S1P concentrations in prepartum plasma were lower in PE controls (787 (722-851) nmol/L) versus normotensive controls (880 (828-934) nmol/L, but the difference was no longer significant after adjusting for gestational age (p=0.105) (**Figure 20C**). The subgroup analysis of PE controls yielded a significant difference in plasma S1P levels between LPE and EPE patients, the latter of whom had lower prepartum S1P, which remained significant after adjustment for gestational age. Compared with prepartum levels, postpartum S1P plasma levels were lower in PE controls, a difference that was significant in LPE but not in EPE patients (**Figure 20D**).

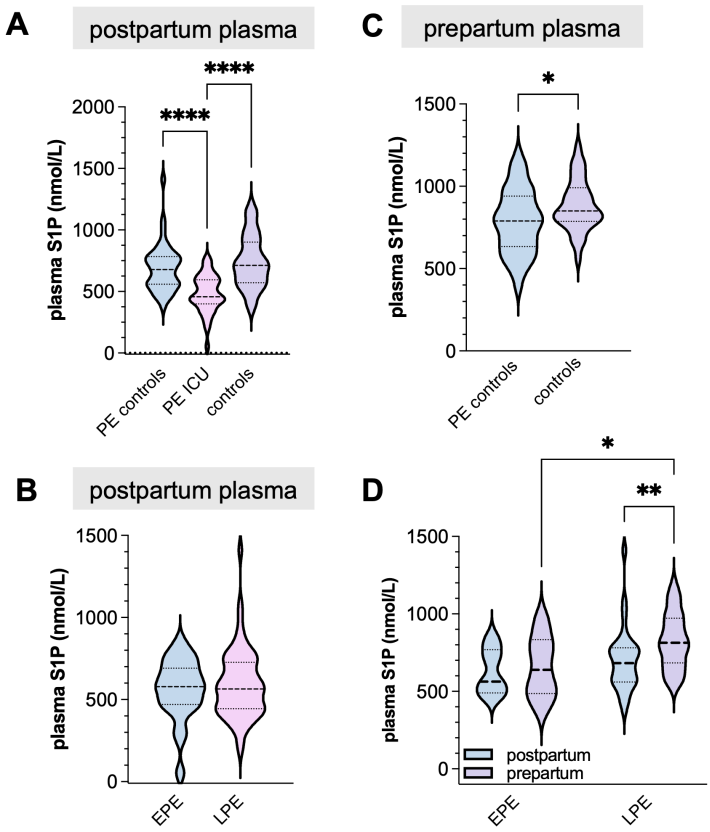

Figure 20
Postpartum plasma sphingosine-1-phosphate (S1P) concentrations in all groups (A) Postpartum S1P concentrations for all PE patients divided in EPE and LPE (B) Prepartum plasma S1P concentrations for PE controls and controls (C) Comparison of prepartum and postpartum plasma S1P concentrations subdivided into EPE and LPE for PE controls (D). * p ≤0.05, ** p ≤0.01, *** p ≤0.001, **** p ≤0.0001. Edvinsson et al. [189].

Placental S1P concentrations are higher in EPE compared with LPE

Placental tissue S1P concentrations were determined for 35 PE controls and 35 normotensive controls. There was no significant difference between the PE (0.60 (0.48-1.1) pmol/mg) and normotensive controls (0.65 (0.49-0.79) pmol/mg). However, significantly higher placental S1P concentrations were observed in the EPE group – a difference that was no longer significant after adjusting for gestational age.

Levels of glycocalyx degradation products are higher in plasma of PE patients

Plasma levels of glycocalyx degradation products were measured in postpartum plasma for all groups.

Postpartum plasma HA concentrations were significantly higher in the ICU cohort (442 (132-788) ng/mL) and in the PE controls (191 (91-454) ng/mL) compared with normotensive controls (102 (77-159) ng/mL). As for HA, plasma SDC-1 levels were highest in the ICU cohort (75 (42-125) ng/mL) versus PE controls (45 (27-66) ng/mL) and normotensive controls (41 (25-69) ng/mL). After adjustments for gestation days, kidney function (PE controls and ICU cohort), and bleeding at delivery, the difference between the ICU-cohort and normotensive controls remained significant.

In contrast, HSPG2 plasma concentrations were lower in the ICU cohort (1.76 (0-3.2) ng/mL) than in PE controls (53.6 (40.2-70) ng/mL, $p<0.001$) and normotensive controls (41.7 (32.5-50.8) ng/mL, $p<0.001$).

The subgroup analysis showed no significant difference in any glycocalyx degradation product between EPE and LPE patients.

Correlations between biomarkers and clinical markers for disease severity

Hemopexin, correlated positively with plasma S1P and plasma HSPG2 and inversely with systolic and diastolic blood pressure, and plasma HA. No significant correlations were found for A1M.

An inverse relationship was observed between plasma S1P and diastolic blood pressure and plasma HA, whereas plasma HSPG2 had a positive association with plasma S1P. Plasma S1P did not correlate with ASAT, uric acid or sFlt-1 after adjustments for multiple comparisons. However, sFlt-1 correlated positively with SDC-1.

The Sequential Organ Failure Assessment (SOFA) score was documented in each patient in the ICU cohort on admission to the ICU. The maximum assigned SOFA score was 7. Plasma Hpx and S1P were lower in patients with higher SOFA scores.

The correlations between all variables are shown in **Figure 21**.

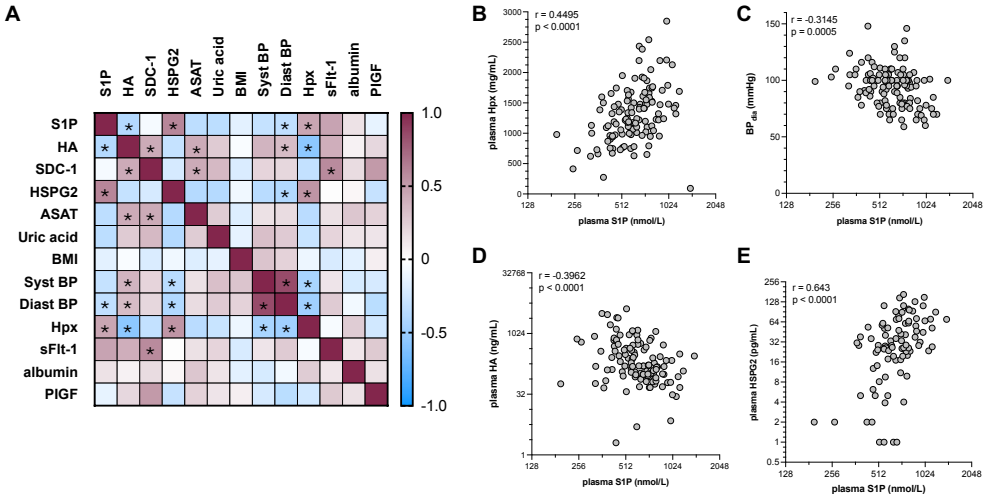

Figure 21
Correlation matrix of the associations between all variables, with significant associations highlighted after correction for multiple comparisons (A), Correlation between postpartum plasma concentration of S1P and Hpx (all groups n=121) (B), Correlation between postpartum plasma concentration of S1P and diastolic blood pressure (all groups n=121) (C), Correlation between postpartum plasma concentration of S1P and postpartum plasma concentration of HA (all groups n=121)) (D), Correlations between postpartum plasma concentration of S1P and postpartum plasma concentration of HSPG2 (all groups n=121) (E). S1P – sphingosine -1-phosphate, Hpx – hemopexin, HA – hyaluronic acid, HSPG2 – heparan sulfate proteoglycan 2. Edvinsson et al. [189]

Summary of Papers I and III

The postpartum concentrations of the biomarkers Hpx, A1M and S1P were lower in severe disease with intensive care need, whereas the glycocalyx degradation products HA and SDC-1 were higher. Intensive care patients, however, harbored decreased plasma concentrations of HSPG2. Plasma Hpx and S1P correlated inversely with clinical disease severity markers such as blood pressure and SOFA scores.

Paper II

Characteristics of the study populations

The distribution of EPE and LPE amongst the PE patients was the same as in Papers I and III, 22.5 % EPE in PE controls and 34 % EPE in the ICU cohort.

Intensive care patients had significantly higher prepartum ASAT, ALAT, creatinine, and uric acid levels compared with the PE controls. Because uric acid varies with gestational age, a z-score was calculated for uric acid in the ICU cohort and PE controls, based on reference values in Lind et al. [207]. The median and mean

z-scores in the ICU cohort were approximately 4 standard deviations above normal values, and approximately 2 standard deviations above normal values in PE controls.

Prediction model for maternal outcome – i.e., need for intensive care

After model development, as described in the methods, the best model was generated when incorporating the variables ASAT, uric acid and BMI, resulting in a cross-validation accuracy of 0.88 and an area under the curve (AUC) of 0.91.

This model was validated internally on the test set, yielding a lower accuracy of 0.82, and an AUC of 0.85. The cross-validated ensemble prediction resulted in an accuracy of 0.92, and an AUC of 0.90.

Neonatal outcome

Patients in the ICU cohort delivered earlier, at 250 (225–267) gestational days compared with 273 (256–278) in the PE controls ($p=0.002$). Also, the neonatal birthweight in the ICU cohort was significantly lower at 2257 g (1949–2565) compared with 3043 g (2720–3366) in the PE-controls ($p<0.001$). However, birthweight was not adjusted for gestational age. For the PE groups, there was no difference in Apgar score at 1 or 10 minutes, but at 5 minutes, scores were significantly lower in the ICU cohort.

Summary of Paper II

There were no clinically relevant differences in neonatal outcome between groups, based on Apgar score at delivery. The prediction model for maternal need of intensive care, showed that ASAT, uric acid, and BMI were the most important predictive factors for an adverse outcome in PE patients. The model performed well when validated internally on a subset of the data.

Paper IV

Characteristics of the study populations

In the PRE-HEART study, a new control group of normotensive pregnancies was introduced, for reasons explained in a previous section. Further, a subgroup of the ICU-patients with the most severe disease (HELLP, eclampsia, or severely dysregulated blood pressure) was selected. Maternal and fetal characteristics for both study groups are shown in **Table 3.** The median stay in the ICU department was 1 day (range 1–3 days). As seen in previous studies, ICU patients had higher

systolic and diastolic blood pressure than normotensive controls, lower fetal birthweights, and more often earlier or premature deliveries. There were no cases of recurrent PE amongst the ICU patients.

At the time of the study, there were no significant differences in blood pressure between groups. Only 1 woman among prior PE cases was diagnosed with chronic hypertension – with ongoing antihypertensive treatment but normalized blood pressure.

Table 3
Characteristics of the women included in the PRE-HEART study at time of delivery and at the time of the current study.

	Patients (n=10)	Controls (n=10)	p value
At time of delivery			
Age (years)	33 [26–40)	34 [31–37]	0.73
Maternal body mass index (kg/m^2)	25.5 [20.0–32.0]	30.0 [25.4–32.8]	0.33
Parity before index pregnancy (n)	0 [0–1]	1 [0–3]	**0.02**
Gestational age at delivery (days)	250 [228–261]	284 [276–291]	**<0.001**
Birthweight (g)	2153 [1515–2447]	3779 [3403 – 4079]	**<0.001**
Highest systolic blood pressure (mmHg)	166 [155–173]	116 [107–123]	**<0.001**
Highest diastolic blood pressure (mmHg)	108 [91–123]	75 [66–78]	**<0.001**
At time of current study			
Time since delivery (years)	5.5 [4.8–7.0]	6.0 [5.0–6.3]	0.59
Age (years)	38 [32–47]	40 [37–44]	0.62
Maternal height (cm)	164 [159–167]	165 [163–171]	0.32
Maternal weight (kg)	67 [61–79]	68 [59–85]	0.84
Maternal body mass index (kg/m^2)	25.1 [22.6–30.2]	25.0 [22.2–30.3]	0.97
Highest systolic blood pressure (mmHg)	111 [107–120]	116 [112–129]	0.14
Highest diastolic blood pressure (mmHg)	68 [63–81]	71 [64–81]	0.67

Values are median [interquartile range] except for parity, which is median (range).

Echocardiography

Previous PE patients have a higher E:A ratio

The E:A ratio (peak velocity blood flow during early filling versus peak velocity flow during atrial contraction) was higher in women with prior PE compared with controls (1.6 vs 1.2; p=0.04). However, no participant fulfilled the established clinical criteria for diastolic dysfunction [199]. Considering the other diagnostic parameters for diastolic dysfunction in the 2 participants with an E:A ratio >2, both had a normal E:e´ ratio (< 8), whereas 1 of them had a mitral deceleration time of > 240 ms (296 ms). Echocardiography data are shown in **Figure 22**.

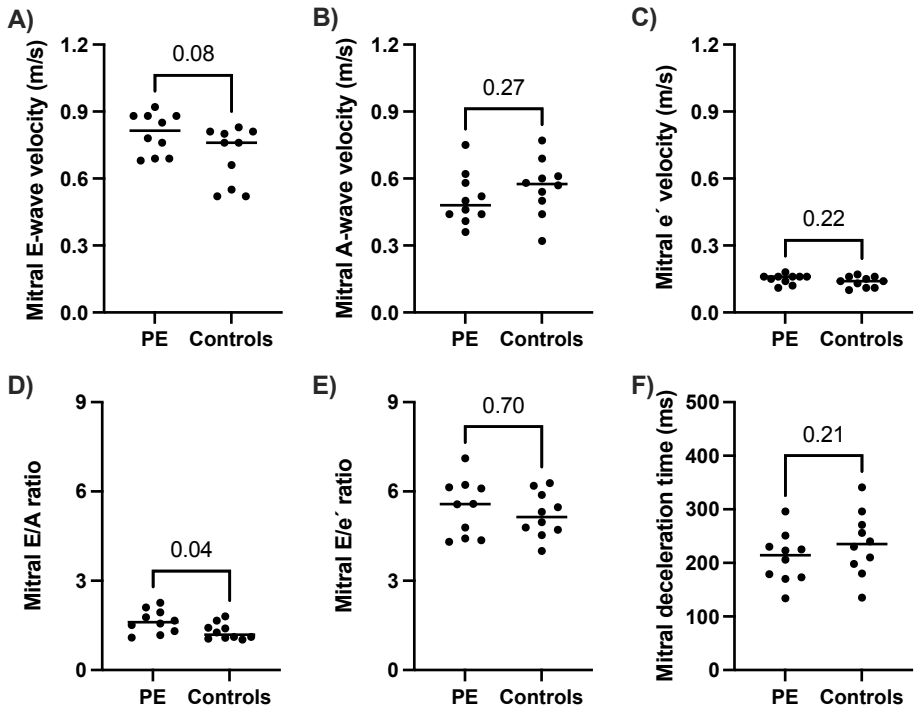

Figure 22
Patient echocardiography data. Mitral E-wave velocity, in m/s (A), Mitral A-wave velocity, in m/s (B), Mitral e´velocity, in m/s (C), Mitral E:A ratio (D), Mitral E:e´ratio (E), Mitral deceleration time, in m/s (F). A p-value of ≤ 0.05 was considered significant. Unpublished manuscript.

Cardiac magnetic resonance imaging

No differences in CMR-acquired parameters between prior PE patients and controls

There were no statistically significant differences between groups with regards to left ventricular or right ventricular volume, left ventricular mass, cardiac index, ejection fraction, or thoracic pulse wave velocity. Cardiac magnetic resonance imaging data are shown in **Figure 23**.

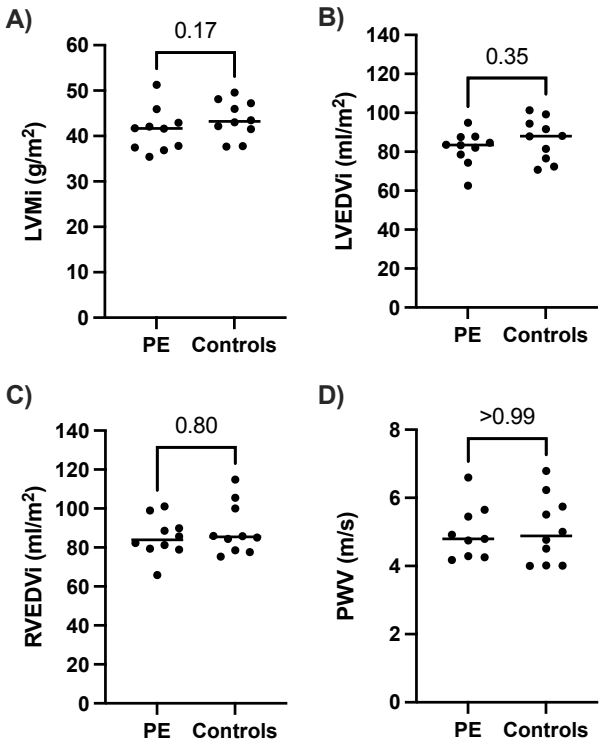

Figure 23
Patient CMR data. Left ventricular mass, indexed values (A), Left ventricular end diastolic volume, indexed values (B), Right ventricular end diastolic volume, indexed values (C), Pulse wave velocity, in m/s (D). A p-value of ≤ 0.05 was considered significant. Unpublished manuscript.

Summary of Paper IV

Women with prior severe PE did not show any changes in cardiac anatomy or function by CMR. Nor did they show increased aortic stiffness as increased PWV. However, a significantly higher E:A ratio was observed by echocardiography in prior PE, but no patient fulfilled the clinical diagnostic criteria for diastolic dysfunction.

Discussion

Papers I-IV

One of the primary aims of this thesis was to describe the characteristics of severe PE in obstetric patients who need postpartum intensive care due to multiorgan failure. The findings in Papers I and III add to and expand previous knowledge on the pathophysiology of PE and unite the complex signaling pathways that regulate oxidative stress, angiogenic imbalance, and vascular inflammation and damage. The vascular endothelium is central in the pathophysiology of PE. Thus, future cure for PE will likely comprise a multifaceted treatment strategy that targets the endothelial dysfunction that is caused by, for example, placental ischemia, oxidative stress, and inflammatory cytokines. The indirect signs of vascular damage, as seen in Paper III, may also reflect the initial process of atherosclerosis, leading to future CVD, which may strengthen the view of PE as an independent cardiovascular risk factor. The link between PE and future CVD was examined further in Paper IV, in which the lack of clinically significant findings may be explained by the absence of persistent hypertension in the participants, in combination with the imaging modalities used.

Pathological routine clinical and biochemical markers, represents the involvement of different organs in PE. However, it is difficult to select the routine biomarkers and vital parameters to monitor specifically for detecting clinical deterioration and thus make the decision to deliver at the right time. The machine learning model that was developed in Paper II could be considered as an example of how a clinical tool for helping decision-making in obstetrics can evolve with AI technology, and how a good prediction can be obtained by including routine parameters.

Biomarkers showing new aspects in the pathophysiology of severe PE

Based on the results of the papers in this thesis, several novel aspects of the pathophysiology of severe PE can be proposed. In Paper I, the ICU cohort bore lower plasma concentrations of Hpx and A1M compared with PE controls. Whereas lower values of Hpx were expected based on previous studies, the decrease in A1M was unanticipated.

Alpha-1-microglobulin is a protein with antioxidative properties [104], and higher levels of A1M have been described in PE pregnancies, as well as in high-

risk pregnancies before onset of the syndrome [110]. These findings suggest a model in which A1M is upregulated during a state of oxidative stress to reduce endothelial and tissue damage in PE. Also, treatment with recombinant A1M has been shown to ameliorate symptoms in animal models of PE [112, 208]. The lower values of A1M in severe disease were thus concluded to be a consequence of an impaired response to the oxidative stress in PE, perhaps explaining the progression of multiorgan involvement. There were no significant correlations between A1M and other biomarkers, implicating this marker as more independent compared with the others.

Lower values of Hpx in PE have been described in previous studies [108, 109] as a sign of consumption in a state of heme-generated oxidative stress, but in Paper I, a graded response that was related to severity was observed. Further, Hpx appears to correlate with blood pressure levels [108], possibly owing to its potential to downregulate angiotensin II receptor expression [119, 120]. Depletion of Hpx is believed to contribute to vasoconstriction and increases in SVR. The lower levels of Hpx in severe disease, as seen in the ICU cohort, may thus explain in part the insufficient control of blood pressure in these patients, who had higher systolic and diastolic blood pressure, despite more intensive treatment with antihypertensive drugs. Heme oxygenas-1 (HO-1) is an enzyme that is activated by Hpx [114] and can be speculated to unite the pathways of angiogenic imbalance, and oxidative stress. Heme oxygenas-1 converts heme to biliverdin, which is then converted by biliverdin reductase to bilirubin and the vasodilator carbon dioxide [209]. It diminishes the oxidative stress in PE through decreased production of reactive oxygen species [209] and heme degradation. Beyond these effects, HO-1 may also influence the release of sFlt-1, and thus have the potential to normalize the angiogenic imbalance in PE. Lower levels of Hpx could therefore exacerbate this angiogenic imbalance, as a result of less attenuation of sFlt-1 [114] and impaired defense against oxidative stress.

The sFlt-1:PlGF ratio was analyzed only in the ICU cohort, but exceeded the gestation specific cut-offs of >85 and > 110, respectively, despite being postpartum values. Although the cure for PE is delivery of the fetus and placenta, this clinical syndrome does not resolve immediately, as reflected by the presence of aberrant biochemical markers and pathological vital parameters several days postpartum, including higher sFlt-1:PlGF ratios. Further, sFlt-1 contributes to the vasoconstriction and higher SVR in PE through upregulation of endothelin-1, increased sensitivity to angiotensin II, and decreased synthesis of NO [129, 130, 132]. This anti-angiogenic marker also promotes vascular damage by binding to heparan sulfate in the glycocalyx [70]. In Paper III, a correlation between sFlt-1 and increased levels of SDC-1 was observed, which may be indicative of a disruption in the structural integrity of the endothelial barrier, given the essential nature of HSPG2 in maintaining vascular integrity [210]. Loss of bound heparan sulfate promotes SDC-1 shedding by increasing its susceptibility to degradation [211].

The main biomarker that was investigated in Paper III, however, was S1P, a sphingolipid with several functions, involved in the development of PE, as well as vascular inflammation and CVD. Plasma S1P was lower in severe PE correlating inversely with blood pressure and plasma HA, and positively with plasma concentrations of HSPG2 and Hpx. A relationship was also observed between lower S1P and clinical severity in the ICU cohort measured as higher SOFA scores. These findings indicate that plasma S1P may serve as a marker for the severity of PE, reflecting the vascular dysfunction that is caused by derangement of the glycocalyx, vasoconstriction and inflammation [149]. Sphingosine-1-phosphate may also have an important function in regulating the response to oxidative stress and ROS by mitigating endothelial dysfunction through the endothelial NOS pathway, generating NO [212]. Reactive oxygen species downregulate SphK1 and thus decrease the levels of S1P [212]. The lower plasma concentrations of S1P in severe PE may be a consequence of oxidative stress, but also contribute to the impaired defense against the oxidative stress in PE.

In summary (**Figure 24**), the mutual protective mechanisms of A1M, Hpx and S1P appear to interact to ameliorate the impact of sFlt-1, oxidative stress, and inflammation on endothelial function in PE. Also, elevated uric acid and obesity increase oxidative stress [213] and inflammation [214], meriting the prominence of these factors in the prediction model for intensive care need in PE, as discussed below. Alpha-1-microglobulin, Hpx and S1P correlate with disease severity, confirming their importance as biomarkers that are involved in the pathophysiology of PE. The signaling pathways of these markers are interesting targets for the development of treatments for PE, as discussed in Future Perspectives.

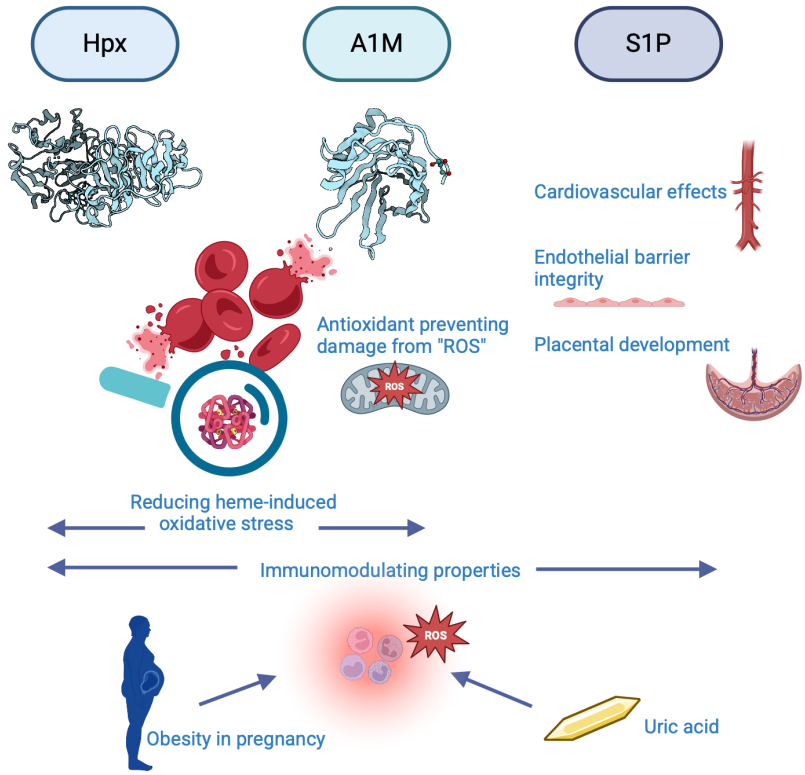

Figure 24
Summary of the protective effects of hemopexin (Hpx), alpha-1-microglobulin (A1M) and sphingosinge-1-phosphate (S1P) and their intertwining pathways. The impact of uric acid and obesity is also shown, leading to inflammation and increased production of reactive oxygen species (ROS). Figure created in Biorender.com.

Cardiovascular assessment

Preeclampsia may be seen as an early warning sign of future CVD. The underlying mechanisms of PE, including vascular damage, inflammation, endothelial dysfunction, metabolic alterations, and hypertension, form a pathway that increases the likelihood of developing cardiovascular conditions later in life, such as heart disease, stroke, and hypertension. The exact pathophysiological mechanism by which PE leads to CVD is unknown. However, one could speculate that the indirect signs of vascular damage, observed as increased levels of glycocalyx degradations products, as shown in Paper III, constitute the initial step in the development of atherosclerosis. A similar process has been suggested in sepsis that is associated with increased CVD-risk [215]. Further, in addition to higher levels of glycocalyx degradation products in the plasma of EPE patients, Weissgerber et al. reported a

smaller width of the glycocalyx, measured as a larger perfused boundary region by sublingual capillaroscopy [75].

In a recently published study by Jälmby et al., glycocalyx thickness was evaluated by sublingual dark-field microscopy 6 years postpartum, showing reduced thickness in previous PE cases compared with normotensive controls. This finding could implicate irreversible damage to the glycocalyx, eventually leading to the development of CVD [216].

In Paper IV, no difference in cardiac structure or function was detected by CMR in PE compared with normotensive pregnancies 4–7 years after their index pregnancy. Cardiac magnetic resonance imaging is the standard modality for evaluating cardiac anatomy and function. Several studies have reported significantly higher LVM in previous PE compared with normotensive pregnancies [217-220]. However, on closer evaluation, increased LVM was associated with persistent hypertension in several of the studies [218-220]. This link has a logical explanation given that cardiac hypertrophy is a compensatory mechanism that is elicited in response to the increased afterload in hypertension. Also, considering the specific values for LVM in Boardman et al. [217] all patients – including those with PE – had normal indexed LVM based on their reported statistical distribution. Further, in Hauge et al. [220] indexed LVM overlapped between women with prior PE and controls. The conclusion that can be drawn from these findings is that LVM may be an unspecific measurement that is related primarily to persistent hypertension.

Cardiac remodeling has been described in PE, as well as in normal pregnancy. However, compared with normal pregnancy there is a trend towards more concentric remodeling in PE, instead of eccentric remodeling [47]. The preponderance of vasoactive substances, such as angiotensin II and endothelin-1, together with inflammatory cytokines, such as IL-6, is considered to be pro-fibrotic, affecting the cardiac collagen network and increasing the risk of cardiac fibrosis [221]. Women with PE have higher levels of these pro-fibrotic cardiac markers during pregnancy and postpartum [222]. There is an established relationship between cardiac remodeling and diastolic dysfunction, which has been shown to occur more frequently in women after PE [223]. In the PRE-HEART study (Paper IV), no gadolinium contrast was given during the CMR procedure. The addition of contrast would have made it possible to evaluate the myocardium and the eventual occurrence of signs of cardiac fibrosis or myocardial scarring. Notably, women with prior PE had significantly higher E:A ratios, which can be a sign of impaired relaxation due to cardiac fibrosis. Thus, the clinical criteria for diastolic dysfunction were not fulfilled. Further, in most cases, cardiac fibrosis is associated with cardiac hypertrophy, which was not observed in any patient group in Paper IV. The relationship between hypertrophy and cardiac fibrosis has been confirmed up to 8 months after pregnancy in an animal model of PE using STOX-1 mice. Indirect signs of pathological cardiac function could be seen by dobutamine stress test at 3- and 6-months post-pregnancy [224, 225].

Despite the negative findings in Paper IV, a strong association between PE and future CVD remains, as shown in the SCAPIS-study [88]. However, routine examinations, such as volumes and function measurements by CMR or echocardiography, as used in the current study, appear to be insufficient to detect cardiovascular changes in the absence of persistent hypertension in an individual patient after PE.

Minhas et al. have observed impaired macrovascular and microvascular coronary endothelial function by CMR, in combination with elevated levels of antibodies against the angiotensin II receptor 1 in postpartum PE women 3 and 6 months after delivery [226]. Macrovascular function was assessed by measuring cross-sectional vessel areas, and microvascular function was measured as changes in blood flow during stress test. Of the included women, 21 % had superimposed PE, causing repeated vascular injury compared with those who experienced a single episode of PE. Impaired coronary microvascular function after PE was also seen by cardiac positron emission tomography within 4 weeks postpartum [227]. The PRE-HEART study, however, did not investigate vascular function at the microvascular level.

Regarding elevated levels of antibodies against the angiotensin II receptor 1 in Minhas et al., it is unknown how long these antibodies persist postpartum. Nevertheless, when present, they activate this receptor, leading to vasoconstriction and hypertension. Future studies should establish whether these antibodies remain for longer periods. If such increases in antibody levels endure, a convenient option would be to treat women with prior PE with angiotensin II receptor blockers, titrating the dose in relation to the blood pressure levels. Even a low-dose regimen may have a positive impact on vascular status.

The cause-and-effect pathways between PE and future CVD remain undefined, and clinically applicable measurements are lacking to detect cardiovascular changes before the onset of symptoms of CVD. Future studies should focus on microvascular assessments based on findings of altered microvascular function by CMR, as well as glycocalyx reduction by sublingual capillaroscopy several years postpartum.

Prediction model

According to a recently published review by Bucher et al., there are no reliable prediction models for adverse outcomes in PE [186]. The only externally validated model is full-PIERS, which has shown moderate accuracy. Angiogenic biomarkers have also yielded promising results but require further validation.

The machine learning-based prediction model that was developed in Paper II, showed that the combination of the parameters ASAT, uric acid and BMI had the highest predictive potential for intensive care need in PE. All included routine parameters in the model development represented objective signs of the diverse organ involvement that occurs in PE, combined with individual patient characteristics.

High BMI correlates robustly with PE – both severe and mild disease – likely mediated by inflammatory pathways and triglycerides [228-230]. In fact, adipose

tissue produces adipokines with functions that resemble those of inflammatory cytokines, such as IL-6. Adipokines also induce the production of ROS and contribute to systemic oxidative stress [214]. Moreover, a higher BMI is generally associated with increased obstetric and anesthesiological risks, despite a pregnancy that is otherwise being considered as normal.

Uric acid is involved in the pathogenesis of PE, through attenuation of normal trophoblast invasion and vascular remodeling of the spiral arteries. Elevated levels usually precede the onset of clinical symptoms [231]. A higher serum uric acid: creatinine ratio has also been shown to be predictive of adverse maternal pregnancy outcomes in PE and thus perhaps constitutes a more specific marker than uric acid alone [232]. Uric acid is generated through the purine synthesis pathway, starting with the breakdown of purines, catalyzed by xanthine oxidase [233]. Xanthine oxidase activity rises in obesity, smoking, liver dysfunction, and in hyperuricemia [233]. Notably, hyperuricemi that is related to increased xanthine oxidase activity leads to ROS production, oxidative stress, and, consequently, endothelial damage [213, 233].

Aspartate aminotransferase is present in all tissues except bone, with the highest levels in skeletal muscle and liver. In hepatocellular injuries, ASAT and ALAT are elevated, the extent to which is usually greater for the latter due to its longer half-life and because a larger fraction of ASAT is bound to mitochondria. The elevated serum level of ASAT in PE is explained by the effect of hypoxia on the liver [234]. Elevated liver enzymes are included as a diagnostic criterion for severe PE and the risk of adverse pregnancy outcomes in PE (PIERS). However, high levels of transaminases are not independently associated with an increased risk of adverse maternal or neonatal outcomes [235]. Uric acid and ASAT were linked to the cardiovascular biomarker S1P in Paper III, although these significant correlations did not persist after adjustments for multiple comparisons.

It is important to emphasize that despite the individual impacts of BMI, uric acid, and ASAT on the pathophysiology of PE, no single pathological parameter reflects an individual patient's risk of adverse outcomes in PE, according to the prediction model in Paper II—rather, it is this triad of variables in the final model development that does so. Based on the results of Paper II, the combination of BMI, ASAT, and uric acid appears to be the parameter to which clinicians should devote extra attention when treating PE patients.

Although the model was validated internally on a test set, performing well with an AUC of 0.85, it nevertheless requires external validation in a new population to be considered sufficiently reliable to be applied in clinical practice.

Strengths

Compared with previous studies, this thesis has detailed the categorization of PE patients, and the proportion of severe PE that required intensive care was larger, contributing novel information on this specific group of patients.

In all studies that entailed measurements of biomarkers, adjustments were made for confounding factors, such as gestational age, kidney function, and blood loss at delivery. In Paper III, the impact of storage time and freeze-thaw cycles was also considered.

Limitations

Limitations concerning the methods that were used in Papers I-IV have been discussed in "Methodological considerations". The small size of the cohorts is a limitation that spans over all the included studies. Also, the retrospective approach in Papers I-III can be viewed as a drawback. Further, no study submitted a pre-registered research protocol.

No power calculation was performed in Paper I or Paper II. However, in Paper III, the effect sizes of previous studies on blood pressure and vascular function to estimate a detectable difference were considered. In Paper IV, the number of participants was based on sample size calculations in Bellenger et al.[203], taking into account the high precision of CMR, combined with experience from previous CMR measurements after pregnancy that has been complicated by PE.

The focus in all of the studies was maternal outcome, not neonatal outcome. Need for neonatal intensive care, neonatal morbidity, and length of hospital stay were not evaluated. Also, there was a higher rate of Cesarean section with general anesthesia in the ICU cohort. No adjustments were made for mode of anesthesia regarding Apgar scores at delivery.

Conclusions

I. Intensive care patients have more clinical risk factors and lower plasma concentrations of Hpx and A1M, suggestive of depletion and thus a reduced capacity to respond to oxidative stress.
II. The postpartum sFlt-1:PIGF ratios in the ICU cohort were consistent with predelivery levels that were predictive of adverse pregnancy outcomes.
III. The clinical routine parameters with the best predictive potential for need for intensive care were ASAT, uric acid, and BMI.
IV. After delivery, intensive care patients harbor lower plasma S1P levels and elevated levels of the glycocalyx degradation markers HA and SDC-1, indicating greater endothelial injury and glycocalyx shedding.
V. Significant correlations were observed between postpartum plasma S1P and HA, Hpx, HSPG2, and diastolic blood pressure. Lower plasma S1P levels were associated with higher SOFA severity scores on admission to the ICU. Thus, S1P is a potential biomarker for endothelial injury and PE severity.
VI. A higher E/A ratio after PE could be a sign of impaired left ventricular relaxation. However, no participant fulfilled the clinical criteria for diastolic dysfunction, after consideration of all variables.
VII. There was no difference between previous severe PE and controls in cardiac anatomy or function by CMR, which may be due to the lack of persistent hypertension in both groups.

Future perspectives

While the aims of this thesis were being developed, approximately 180 women had been treated in intensive care units in Sweden annually due to PE (SIR). Some hospitals also have high-dependency units, but national data on the number of PE patients who require this type of care each year remain lacking. In Sweden, mortality in PE is decreasing, likely due to updated guidelines and improved care. Consistent with these patterns, it can be speculated that the need for intensive care for this group of patients will decrease.

Statistics from SIR, show that the number of days in the ICU for patients with PE, HELLP or eclampsia declined from 184 in 2016 to 117 in 2023. Notably, fewer days for PE patients in the ICU were already registered during the Covid pandemic, likely attributed to a lack of resources. The Swedish Intensive Care Registry recently reported a 5% loss in ICU beds from 2019 to 2024. The ongoing shortage of ICU beds, in combination with fewer days registered for PE patients in the ICU, may be explained in part by PE women being less prioritized compared with other patients` categories – fortunately, without an increase in short-term mortality as a consequence. This aspect of there being fewer PE patients in intensive care could render evaluation of the prediction model (Paper II) in a larger external cohort more difficult if the same outcome measure is used. To this end, national cooperation and inclusion of all ICU patients in Sweden during a few years could make such an assessment possible.

In developing a cure for PE, it would be useful to focus on how interventions could specifically target the complex pathophysiological pathways that lead to endothelial damage and the other associated hallmarks of this syndrome. Pre-eclampsia is a multi-faceted condition, and any treatment would need to address these interconnected mechanisms on multiple levels.

Therapeutics that are aimed at directly reducing oxidative stress could play a pivotal role. Alpha-1-microglobulin has shown therapeutic potential in ameliorating PE symptoms in animal models, and in reducing kidney injury. The A1M protein is being evaluated in Phase II trials for kidney injury during heart surgery as the indication [236]. Other potential indications are preservation of kidney function after transplantation and protection of the kidney in sepsis. Historically, pregnant patients have been excluded from the development of pharmaceuticals for pregnancy-related complications due to fear of harming the fetus. Research on pregnant patients thus remains plagued by ethical concerns with regard to the development of new treatment methods. If a drug can be shown to be safe in another

context or remain behind the placental barrier despite the endothelial damage seen in PE, the possibilities for studies in pregnant women could expand. If A1M is considered safe in clinical trials in nonpregnant individuals, it may thereafter be evaluated in PE cases, hopefully improving outcomes for women and their unborn children, as seen in animal models.

The sFlt-1:PlGF ratio has generated promising results as a predictive biomarker and is continuously being validated in clinical settings. This ratio requires further evaluation in Swedish healthcare before it can be used routinely. Drugs or biologics that specifically inhibit sFlt-1 activity could counteract its negative impact on endothelial cells. Targeting sFlt-1 with monoclonal antibodies or small molecules that block its binding to VEGF and PlGF might reduce its anti-angiogenic effects, thus improving endothelial function. Removal of sFlt-1 by apheresis has been tested in a pilot study, but this approach needs further evaluation to determine its safety and pregnancy-prolonging potential. A novel siRNA drug, CBP-4888, that is delivered subcutaneously to suppress sFlt-1 expression in the placenta, has recently obtained fast track approval from the Food and Drug Administration (FDA) and is undergoing a Phase 1 clinical testing [237]. However, the drug is delivered by viral vectors or nanoparticles, raising safety issues as a major concern that must be evaluated further.

Sphingosine-1-phosphate is an interesting potential biomarker of severity in PE. S1P signals through S1PR 1–3, and targeting these receptors could mitigate the vascular and inflammatory components of PE. For example, activation of S1PR1 may improve endothelial barrier function and prevent excessive blood vessel leakage, a key feature of PE, whereas blocking S1PR2 might preclude the pathological vascular changes that are associated with PE. Future studies should include larger cohorts but also investigate the receptor binding profiles for S1P to examine the relationship between S1P levels and glycocalyx degradation and their implications for endothelial dysfunction in PE and future CV. Although the potential for targeting S1P signaling as a treatment for PE is promising, further research is required to validate safety and efficacy of such an approach in clinical settings.

Populärvetenskaplig sammanfattning

Preeklampsi, eller havandeskapsförgiftning, drabbar cirka 5 000 gravida kvinnor per år i Sverige. Globalt är dödligheten hög, och cirka 76 000 kvinnor och 500 000 spädbarn mister årligen livet till följd av preeklampsi. I Sverige är dödligheten låg, men varje år blir cirka 180 kvinnor så allvarligt sjuka av sin preeklampsi att intensivvård är nödvändigt till följd av sjukdomskomplikationer. Det finns idag ingen behandling för preeklampsi, enda sättet att ”bota” är genom att avsluta graviditeten genom förlossning. Tidpunkten för detta avgörs utifrån kvinnans medicinska tillstånd. Det är alltid den gravida kvinnans mående som kommer i första hand. I många fall måste förlossning ske innan barnet är moget för att klara ett liv utanför livmodern, vilket innebär att preeklampsi är en betydande orsak till att barn föds för tidigt i Sverige och i resten av världen. Många av barnen är dessutom tillväxthämmande som en följd av en dåligt fungerande moderkaka.

Preeklampsi drabbar inte bara kvinnan i samband med graviditet och förlossning utan sjukdomen har också långtidseffekter på hjärta och kärl, vilka kan visa sig flera år senare.

Orsaken till att preeklampsi uppstår är inte helt känd, men den mest etablerade teorin kallas för ”tvåstegs-modellen” där steg ett utgörs av en defekt utveckling av moderkakan och steg två är kvinnans symptom som uppstår som en följd av organskadan som skett. Som en konsekvens av att moderkakan inte växer in i livmodern på rätt sätt, blir otillräcklig kärlförsörjningen mellan kvinnan och fostret begränsande för blodflödet. Konsekvensen av detta blir en relativ syrebrist i moderkakan och försämrad transport av näringsämnen till fostret. Syrebristen i moderkakan leder till bildandet av fria syreradikaler, ett tillstånd som kallas ”oxidativ stress” uppstår. Fria syreradikaler är nämligen skadliga för både själva moderkakan, och för kvinnans kärl, och bidrar därmed till en form av fysiologisk stress. Syrebristen leder också till att de celler i moderkakan som utgör en barriär gentemot kvinnans blodcirkulation, syncytiotrophoblasterna, släpper ut cellfragment och blåsor (vesiklar) i kvinnans cirkulation, vilka i sin tur orsakar ytterligare ”oxidativ stress”, inflammation samt en generell kärl- och organpåverkan. Skadorna på blodkärlen leder till att de drar ihop sig, blir mer genomsläppliga för vätska, och sannolikt också mer benägna att utveckla framtida åderförkalkning. Konsekvensen av de kärlsammandragande effekterna blir i akutskedet ett högt blodtryck, vilket är det huvudsakliga tecknet på preeklampsi. Förutom ett högt blodtryck får den gravida kvinnan också symptom från andra organsystem. Dessa symptom kan vara njurpåverkan, leverpåverkan, påverkan på

blodets levringsförmåga, kramper, hjärtsvikt eller tecken på att fostret inte växer som det ska. Fostret räknas numera som ett eget organsystem i samband med att kvinnan är gravid.

Det finns två huvudtyper av preeklampsi. Den tidiga varianten som uppstår före graviditetsvecka 34 och den sena som uppstår efter graviditetsvecka 34. Tvåstegsmodellen ovan beskriver främst utvecklingen av tidig preeklampsi. Sent debuterad preeklampsi beror i högre grad på riskfaktorer hos den gravida kvinnan såsom diabetes, övervikt, eller njursjukdom, i kombination med att moderkakan inte har förmåga att upprätthålla sin funktion när utrymmet i livmodern blir för litet i slutet av graviditeten. I de sena fallen uppstår ”oxidativ stress” i placentan alltså senare i graviditeten.

På en intensivvårdsavdelning finns möjligheter att behandla de organkomplikationer som kan uppstå vid en svår preeklampsi. Det finns också resurser för att kontinuerligt övervaka kvinnans tillstånd, vilket inte är möjligt på en vanlig förlossningsavdelning eller BB-vårdavdelning.

Syftet i **delarbete I och III** var att ta reda på mer om de bakomliggande orsakerna till att vissa kvinnor blir så svårt sjuka av sin preeklampsi att de behöver intensivvård, medan andra får ett mindre allvarligt sjukdomsförlopp. Koncentrationen av ett antal faktorer, s.k. biomarkörer, analyserades i blodet hos tre grupper av kvinnor; en grupp med allvarlig preeklampsi som behövde vårdas på intensivvårdsavdelning, en grupp med preeklampsi som vårdades på vanlig förlossningsavdelning och en grupp med friska graviditeter. Generellt är en biomarkör ett ämne som är mätbart, och som återspeglar en biologisk process i kroppen. En ideal sjukdomsmarkör reflekterar sjukdomens svårighetsgrad och är inte mätbar i en frisk population. Resultaten visade att de kvinnor som blir svårt sjuka i preeklampsi hade ett sämre försvar mot den ”oxidativa stress” som uppstår i vid preeklampsi, och även tecken på mer kärlskada. Blodnivåerna av de olika biomarkörerna kan delvis förklara de allvarligare kärl- och organskadorna och därmed reflektera sjukdomens svårighetsgrad.

För att i framtiden kunna bota preeklampsi är det viktigt att ha kunskaper om underliggande sjukdomsmekanismer. En av biomarkörerna som mättes i **delarbete I** är alpha-1-microglobulin som är en viktig antioxidant. En antioxidant motverkar effekten av de fria syreradikalerna och förhindrar uppkomst av ”oxidativ stress”. Denna antioxidant har i djurstudier visat sig kunna förhindra organskador vid preeklampsi. Det har även visat sig att alpha-1-microglobulin kan förhindra njurskador som uppstår i samband med hjärtoperationer orsakade av fritt hemoglobin. Fritt hemoglobin släpps ut från de röda blodkropparna som tar skada i hjärt-lungmaskinen. Nedbrytningsprodukter av fritt hemoglobin bidrar till ”oxidativ stress” och efterföljande njurskador, vilket även ses vid preeklampsi. Det pågår Fas II studier på människor som genomgår hjärtkirurgi, men ännu sker inga studier med detta potentiella läkemedel på gravida kvinnor med preeklampsi.

Att med hjälp av biomarkörer kunna förutsäga vilka kvinnor som riskerar att utveckla en allvarligt preeklampsi hade varit till nytta för att veta vilka som i sin tur

är i behov av en tätare övervakning och eventuellt behöver genomgå förlossning i ett tidigare skede. Det tas många blodprover och blodtryckskontroller på kvinnor som vårdas för preeklampsi, men det är svårt att veta vilka av alla dessa provsvar som har störst betydelse för att förutsäga risken för svår sjukdom. I **delarbete II** användes en form av artificiell intelligens som kallas för ”maskininlärning” i syfte att skapa en modell för att förutsäga eller prediktera allvarlig sjukdom. Kvinnornas blodprovsvärden, även kallade variabler, matades in i modellen, som utvecklades genom att träna programmet på att hitta den bästa kombinationen av variabler för svår sjukdom. Efter att modellen bearbetat alla data, framstod tre variabler som mest betydelsefulla för att utveckla allvarlig sjukdom. Dessa var patientens vikt speglad som ”body mass index” (BMI), leverprovet aspartate aminotranferas (ASAT) samt urinsyra (urat).

Övervikt är en känd riskfaktor för att utveckla en allvarlig form av preeklampsi. Sannolikt beror detta på att en ökad mängd fettvävnad bidrar till en högre grad av inflammation i kroppen, vilket också leder till att blodtrycket hos överviktiga oftare blir högre även under en normal graviditet. Ett förhöjt ASAT är tecken på att levern är påverkad av preeklampsin, vilket främst sker i allvarligare fall. Förhöjda uratvärden kan bero på överaktivitet av ett enzym som ökar hastigheten i den kemiska reaktionen som leder till bildningen av urat. Detta kan också bidra till en ökad ”oxidativ stress” i kroppen samt ett ökat inflammatoriskt tillstånd.

I **delarbete IV** undersöktes om kvinnor som vårdats inom intensivvården för preeklampsi 4–7 år tidigare, nu visade tecken på hjärt-kärlsjukdom jämfört med kvinnor som haft helt normala graviditeter under samma tidperiod. I studien ingick totalt 20 kvinnor, tio med tidigare preeklampsi och tio kontroller. Deltagarna genomgick blodtrycksmätning i vila, ultraljud av hjärtat samt magnetkameraundersökning av hjärtat. Blodtrycksmätningen visade ingen skillnad mellan grupperna, d.v.s. de kvinnor som hade haft en preeklampsi hade inte utvecklat ett kroniskt förhöjt blodtryck i högre grad än de friska kvinnorna. Ultraljud av hjärtat visade att två kvinnor med genomgången preeklampsi hade ett avvikande mått som kan tala för ett stelare hjärta, men när samtliga mått för att ställa diagnosen hjärtsvikt lades ihop, uppfylldes inte kriterierna. Magnetkameraundersökning visade ingen skillnad mellan grupperna avseende hjärtats uppbyggnad och funktion. Avsaknad av skillnader mellan grupperna, tolkades främst bero på avsaknad av högt blodtryck. Högt blodtryck leder till att hjärtat omformas för att anpassa sig till ett högre motstånd i kärlen.

Andra studier som undersökt förändringar i mindre kärl hos liknande patientgrupp har kunnat påvisa permanenta förändringar i kärlens inre lager hos preeklampsipatienter 4–7 år efter förlossning. Med hjälp av ett mikroskop som synliggör blodkropparnas rörelse i de små kärlen under tungan, kapillärer, kunde en permanent kärlskada påvisas.

Sannolikt finns det alltså permanenta kärlförändringar hos patienter med genomgången preeklampsi, men andra undersökningsmetoder för att upptäcka dessa är nödvändiga. Metoderna som användes i delarbete IV upptäcker främst mer

uttalade förändringar på hjärta och större kärl. Att identifiera kärlförändringar och/eller ett förhöjt blodtryck tidigt är viktigt för att kvinnor med genomgången preeklampsi inte ska hinna utveckla en svår hjärt-kärlsjukdom eller ett svårbehandlat blodtryck, hjärtinfarkt, hjärtsvikt eller stroke.

I framtiden är det viktigt att kvinnor med genomgången preeklampsi kallas till en tidig uppföljning, på en specialanpassad mottagning för kvinnor som haft komplicerade graviditeter. Det är viktigt att ge rätt livsstilsråd, och tillhandahålla en fortsatt uppföljning av blodtrycket och värdering av andra kardiovaskulära riskfaktorer. Kvinnorna och deras partners är ofta psykiskt traumatiserade efter en genomgången preeklampsi och behöver även stöd ur dessa aspekter.

Kvinnor med preeklampsi har varit en förbisedd grupp och bör prioriteras mer både i akutskedet samt med avseende på uppföljning av långtidseffekter.

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About the author

CAMILLA EDVINSSON is a consultant in anesthesia and intensive care at Helsingborg Hospital, Sweden. She holds a SSAI certificate in obstetric anesthesia, which is also her research area. This thesis examines the mechanisms involved in the pathophysiology of severe preeclampsia, as well as its long-term cardiovascular outcomes. It also explores the potential of using machine learning models to predict intensive care need in patients with preeclampsia.

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