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Preeclampsia and the brain

The blood-brain barrier and neurological consequences

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Abstract

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Cerebral complications of preeclampsia are among the leading causes of maternal mortality. Women with previous preeclampsia and eclampsia have increased long-term risks of cognitive impairment, stroke, and vascular dementia. They report a lower quality of life, concentration issues, and tiredness after childbirth. The pathophysiology of cerebral complications remains unclear, however, is suggested to involve blood-brain barrier (BBB) impairment, loss of cerebral autoregulation, microinfarctions, and edema.

This translational thesis aimed to explore pathophysiological mechanisms of BBB impairment in preeclampsia and to investigate whether preeclampsia and eclampsia increase the risk of neurological disorders and sick leave in the years following childbirth. This was explored through two preclinical laboratory studies and two register-based cohort studies.

The BBB was explored using an *in vitro* model. Results were correlated to plasma concentrations of cerebral biomarkers. Correlations were estimated with non-parametric tests. Plasma from women with preeclampsia affected the *in vitro* model of the human BBB by increasing permeability to FITC-Dextran and decreasing transendothelial electrical resistance (TEER) at the cellular level. All cerebral biomarkers were increased in plasma from women with preeclampsia, compared with normotensive pregnancy. Increased plasma concentrations of NfL were correlated to a decrease in TEER over the BBB. Plasma concentrations of tau, NSE and S100B were not associated with TEER.

Associations between gestational hypertension, preeclampsia and eclampsia, and a composite of neurological disorders (migraine, headache, epilepsy, sleep disorders and neurasthenia) were estimated with multivariate Cox regression models. All exposure groups were associated with an increased risk of a composite of neurological disorders, compared with normotensive pregnant women. Gestational hypertension and preeclampsia were associated with increased migraine risk. The strongest association was found between eclampsia and epilepsy.

Associations between preeclampsia and sick leave rates in the second year postpartum were assessed with augmented inverse probability weighting. Women with preeclampsia took more sick leave compared with women without preeclampsia.

In conclusion, plasma from women with preeclampsia impairs BBB function *in vitro*, and BBB leakage is indicated by correlation between decreased TEER and increased plasma NfL. Women with preeclampsia, particularly eclampsia, face a higher risk of developing neurological disorders postpartum, which may reflect the increased sick leave observed in this group.

Keywords: Preeclampsia, Eclampsia, Blood-Brain Barrier, Cerebral Biomarkers, Neurological Disorders, Sick Leave

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To my family

Cover illustration by Maria Grudéus.

List of Papers

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

I. Bergman, L.; Acurio, J.; Leon, J.; Gatu, E.; **Friis, T.**; Nelander, M.; Wikström, J.; Larsson, A.; Lara, E.; Aguayo, C.; et al. Preeclampsia and Increased Permeability Over the Blood-Brain Barrier - a Role of Vascular Endothelial Growth Receptor 2. *Am J Hypertens*, 2021 Feb 18;34(1):73-81. *Reproduced with permission from Oxford University Press.*

II. **Friis, T.**; Wikström, A.-K.; Acurio, J.; León, J.; Zetterberg, H.; Blennow, K.; Nelander, M.; Åkerud, H.; Kaihola, H.; Cluver, C.; et al. Cerebral Biomarkers and Blood-Brain Barrier Integrity in Preeclampsia. *Cells*, 2022 Feb 24;11(5):789, 11. *Reprint made under the Creative Commons Attribution License.*

III. **Friis, T.**; Bergman, L.; Hesselman, S.; Lindström, L.; Junus, K.; Cluver, C.; Escudero, C.; Wikström, A.-K. Gestational hypertension, Preeclampsia, and Eclampsia and Future Neurological Disorders. *JAMA Neurol*. 2025;82(2):142-51. *Reproduced with permission from American Medical Association.*

IV. **Friis, T.**; Wikström, A.-K.; Hesselman, S.; Lindström, L.; Junus, K.; Cluver, C.; Escudero, C.; Bergman, L. Associations between Pre-eclampsia and Sick Leave following Childbirth: A Swedish Register-based Cohort Study. *Submitted.*

Contents

Abbreviations

BBB	Blood-brain barrier
BCEC	Brain capillary endothelial cell
BMI	Body mass index
CI	Confidence interval
CNS	Central nervous system
CSF	Cerebrospinal fluid
DAG	Directed acyclic graph
DBP	Diastolic blood pressure
ELISA	Enzyme-linked immunosorbent assay
FITC-Dextran	Fluorescein isothiocyanate-Dextran
hCMEC/D3	Human cerebral microvascular endothelial cell line
HDP	Hypertensive disorders of pregnancy
HELLP	Hemolysis, elevated liver enzymes, low platelets
HR	Hazard ratio
ICD-10	International classification of diseases, tenth revision
IQR	Interquartile range
IUGR	Intrauterine growth restriction
LISA	Longitudinal integrated database for health insurance and labor market studies
MBR	Swedish Medical Birth Register
$MgSO_4$	Magnesium sulphate
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
NfL	Neurofilament light
NSE	Neuron-specific enolase
PCR	Polymerase chain reaction
PRES	Posterior reversible encephalopathy syndrome
SBP	Systolic blood pressure
SD	Standard deviation
Simoa	Single molecule array
SGA	Small for gestational age
SLE	Systemic lupus erythematosus
S100B	S100 calcium-binding protein B
TEER	Trans endothelial electrical resistance
VEGF	Vascular endothelial growth factor
VEGFR	Vascular endothelial growth factor receptor

Introduction

Preeclampsia is a pregnancy-specific disorder that affects 3-5% of all pregnancies. It is one of the most common causes of maternal and perinatal morbidity and mortality.[1] Annually, more than 70.000 maternal deaths are associated with hypertensive disorders of pregnancy (HDP), where cerebral complications, such as eclampsia, cerebral hemorrhage and cerebral edema, are leading causes.[2-4] Long-term consequences after HDP are well described, especially cardiovascular but also to some extent neurological, where increased risks of cognitive impairment, vascular dementia, and stroke later in life have been reported.[5-7] Women with preeclampsia and eclampsia also report lower quality of life, concentration issues, and tiredness years after childbirth.[8]

The pathophysiology of cerebral complications in preeclampsia is not fully understood. Increasing evidence suggest that multiple factors, such as blood-brain barrier (BBB) impairment, loss of cerebral autoregulation, cerebral edema formation, and microinfarctions are involved.[9,10]

This thesis aims to explore the pathophysiological mechanisms behind acute cerebral complications in preeclampsia, if they can be related to circulating biomarkers, and if women who suffered from preeclampsia and eclampsia during pregnancy have lasting neurological effects.

A human *in vitro* model of the BBB may better reflect cerebral pathophysiology in preeclampsia than previously used animal models. If cerebral biomarkers could indicate BBB injury, a blood test may identify women at risk of cerebral complications. Early diagnosis could improve both acute and long-term neurological outcomes for these women.

The impact of acute cerebral complications in women with preeclampsia and eclampsia on medium-term neurological health and sick leave in the years following childbirth is unclear. Increased knowledge in this field may improve obstetric care and postpartum follow-up of these women.

Preeclampsia

Definition

Preeclampsia is defined as hypertension after 20 weeks of gestation accompanied by signs of maternal organ dysfunction.[11]

The traditional definition of preeclampsia required new onset hypertension (systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg) in combination with proteinuria (U-protein ≥ 0.3 g/24 h, a spot urine protein creatinine ratio ≥ 30mg/mmol or albumin creatinine ratio ≥ 8 mg/mmol, or at least 1g/L (2+), or 1+ detected two times on a dipstick test), after 20 weeks' gestation. Historically, these were the first recognizable signs in a pregnant woman, preceding eclampsia.[12]

In 2014, the International Society for the Study of Hypertension in Pregnancy (ISSHP) stated that proteinuria was no longer mandatory for the diagnosis of preeclampsia. Instead, hypertension (new-onset or manifest) can be accompanied by any manifestation of maternal organ dysfunction.[11] The new definition incorporates the diversity and complexity of the syndrome and suggests that preeclampsia may be consisting of several different phenotypes, possibly with different pathophysiological pathways.

In Sweden, the Swedish Society of Obstetrics and Gynecology (SFOG) recommended implementing new diagnostic criteria in clinical praxis starting in December 2019.[13] The new definition included hypertension together with at least one of the following criteria of maternal organ manifestations:

- Kidney impairment with significant proteinuria (≥0.3 g/24 h, albumin/creatinine ratio ≥ 8 mg/mmol, or protein/creatinine ratio ≥ 30 mg/mmol), or acute kidney failure: creatinine > 90 µmol/L, or oliguria (< 500 ml/24 h).
- Liver involvement: Elevated transaminases > 2 times upper reference limit. Persistent epigastric pain or right upper quadrant pain.
- Hematological complications: Rapid decline in thrombocytes, thrombocytopenia (platelet count < 100.000 /µL). Hemolysis with haptoglobin < 25 g/L, lactate dehydrogenase > 600 U/L or > 10.0 µkat/L.
- Neurological symptoms: Severe persistent headache, persisting visual disturbances, positive foot clonus, eclampsia (tonic-clonic seizures in preeclampsia), or stroke.
- Cardiovascular/pulmonary complications: Pulmonary edema, chest pain.
- Uteroplacental dysfunction: Intrauterine growth restriction (IUGR).

The previous definition, which included both hypertension and proteinuria, was still recommended in research settings to ensure diagnostic specificity at the time the studies included in this thesis were planned.[11]

For research purposes, it can be useful to distinguish between preeclampsia without severe features and preeclampsia with severe features. Whilst in a clinical situation, preeclampsia should always be considered as a condition capable of progressing at any time to become severe or even life-threatening for both the mother and the fetus.[11] According to SFOG, preeclampsia with severe features is defined by SBP ≥ 160 mmHg and/or DBP ≥ 110 mmHg, maternal organ manifestations beyond proteinuria, and/or clinical symptoms.[13]

Preeclampsia is often categorized into early-onset (diagnosis before 34 weeks of gestation) and late-onset (diagnosis at 34 weeks or later) preeclampsia. These subtypes are associated with different fetal and maternal risks.[14,15] In register-based research, preeclampsia is typically categorized as preterm (birth before 37 weeks of gestation) and term (birth at 37 weeks or later). This classification arises because the registers usually record the time of birth associated with preeclampsia, rather than the exact time of diagnosis.[16]

Epidemiology

Preeclampsia affects 3-5% of all pregnancies, but great variation is reported throughout the world, with incidence as high as 8% in some regions.[1,17] Preeclampsia is the second most common cause of maternal and perinatal morbidity and mortality worldwide.[1,3] HDPs are responsible for approximately 10-18% of maternal deaths worldwide, which gives an estimation of around 70,000 deaths per year. However, death rates vary considerably across geographical regions.[3]

A severe form of preeclampsia is eclampsia, which in high-income countries is reported at 2 to 3 cases per 10,000 births, while the incidence is estimated to 16-69 cases per 10,000 births in low- and middle-income countries.[17-19] Cerebral complications, such as eclampsia, cerebral edema, and cerebral hemorrhage are among the major causes of death in women with preeclampsia during pregnancy and in the post-partum period.[2,17]

Pathophysiology

In early pregnancy, trophoblast cells invade the uterine decidua and one third of the myometrium. They destroy both the endothelium and the muscular layer of the spiral arteries, resulting in the transformation into greater vessels with less resistance, in order to allow an increased blood flow into the intervillous space. As a response to normal placentation, increased levels of angiogenic

growth factors (e.g., vascular endothelial growth factor (VEGF), placental growth factor (PlGF)) are released into the mother's circulation. These factors are vital for the normal development of the placenta, the intrauterine environment, and the growth and development of the fetus.[20,21]

To date, the pathophysiology of preeclampsia is not completely understood, but increasing evidence indicates that the pathophysiological pathways may differ between early- and late-onset preeclampsia.[1] Early-onset preeclampsia is suggested to include a two-stage process.[22] In the first stage, insufficient invasion of trophoblast cells into the walls of spiral arteries leads to inadequate remodeling of the vessels, which results in narrow and high-resistance spiral arteries. This will restrict blood flow to the placenta and cause hypoxia and ischemia, resulting in impaired placentation.[23] Following this, in the second stage, the under-perfused placenta generates oxidative stress, releasing inflammatory and anti-angiogenic factors into the maternal circulation. This leads to widespread inflammation and progressive endothelial damage, potentially due to an imbalance between angiogenic and anti-angiogenic factors, resulting in clinical symptoms in the mother.[23] The pathophysiology of late-onset preeclampsia has been described by the "placenta in the pot" theory, in which the growing placenta and fetus place increasing demands on oxygen supply throughout pregnancy. With time the placenta outgrows the uterine capacity, which causes mechanical restriction of the intervillous space. This leads to reduced perfusion of the placenta and as a result hypoxic stress arises, followed by the same maternal responses as described in the second stage.[23]

Maternal genetic predisposition to cardiovascular and metabolic diseases like diabetes, obesity, chronic hypertension, and some autoimmune diseases, which are all conditions with excessive chronic inflammation, may influence both the initial development of preeclampsia and the maternal response to harmful substances that are released from the placenta.[21,23] With the physiological burden of pregnancy (even with normal placentation), the increasing vascular inflammation may eventually lead to clinical symptoms of preeclampsia.[23,24] Although early- and late-onset preeclampsia are often considered distinct subtypes, the contributions of placental and maternal factors most likely vary among individuals, resulting in clinical preeclampsia manifesting at different gestational ages.[25] Since early-onset preeclampsia occurs in the first trimester, it leads to prolonged uteroplacental perfusion dysfunction, resulting in a higher incidence of IUGR compared to late-onset cases.[23]

The underlying pathophysiological mechanisms of the association between HDP and long-term cardiac and cerebrovascular consequences are not completely clarified, but the metabolic syndrome, an unfavorable cardiovascular phenotype, and hormonal changes may contribute.

Several of the angiogenic growth factors and inflammatory substances released during normal pregnancy will affect the endothelium throughout the mother's circulation, resulting in increased vascular permeability. In

preeclampsia, with an excess release of these factors, instead of adapting the maternal body to pregnancy, they may cause harm.[26]

Endothelial dysfunction is associated with increased production and release of VEGF, an endogenous mediator which regulates various endothelial functions, including cell proliferation, migration, vascular permeability, and secretion. VEGF binds and activates the tyrosine kinase receptors type 1-3 (VEGFR1-3). Expression of VEGFR2 is mainly found in vascular endothelial cells. When VEGF binds to VEGFR2, it initiates pro-angiogenic signaling. However, when it binds to the soluble VEGFR1 decoy receptor – soluble fms-like tyrosine kinase-1 (sFlt-1) – it exerts anti-angiogenic effects. In preeclampsia, sFlt-1 is upregulated and overexpressed in the placenta, leading to an angiogenic imbalance. This imbalance results in abnormal binding of VEGF and PlGF,[21,27] which may explain the widespread endothelial dysfunction observed in preeclampsia, along with its various clinical manifestations.

Pathophysiology of cerebral complications in preeclampsia

Preeclampsia sometimes results in acute cerebral complications, such as eclampsia, cerebral edema, and cerebral hemorrhage. There are several theories regarding the pathophysiology of these conditions, including impairment of BBB permeability, a loss of autoregulation of blood pressure in the cerebral circulation, and edema formation.[9] Figure 1 illustrates how inflammatory (e.g., interleukins, cytokines) and anti-angiogenic (e.g., sFlt1, sEng) factors released by the placenta in preeclampsia may reach the brain endothelial cells. These may then disrupt the BBB with increased leakage of fluids and substances over the barrier, resulting in intracerebral edema, damage of the brain parenchyma, and eclampsia.[28]

While ischemic strokes are more common overall, pregnancy – especially when complicated by preeclampsia – is associated with a higher proportion of hemorrhagic strokes compared to the general population. Contributing factors include endothelial dysfunction, alterations in the BBB, severe hypertension, and compromised cerebral autoregulation.[29,30]

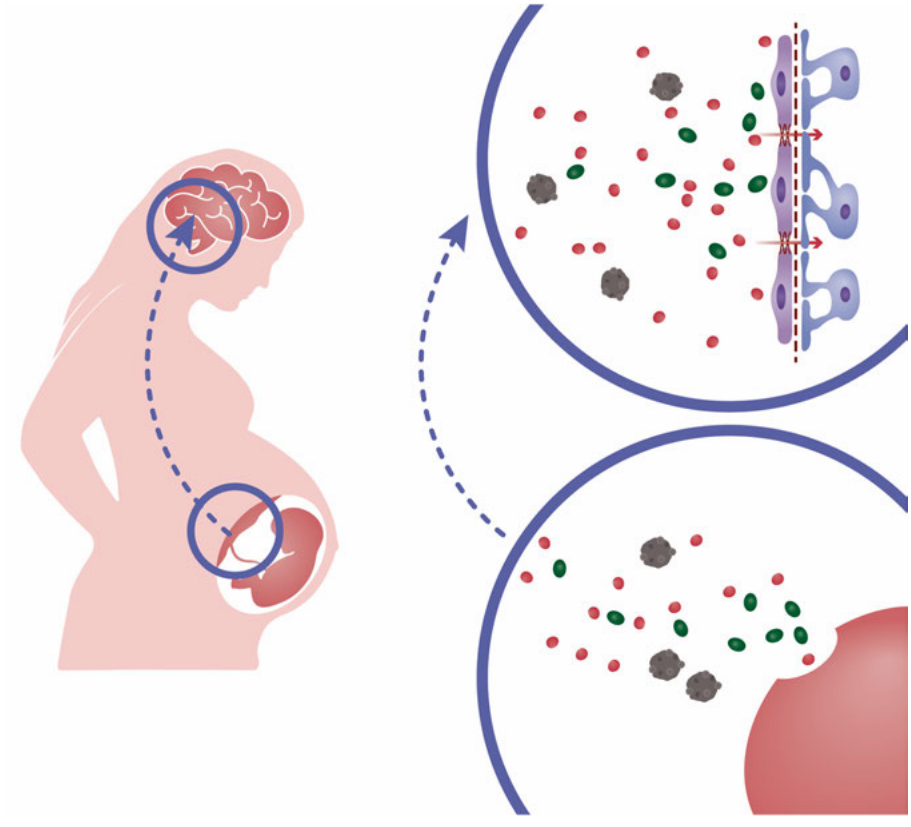

Figure 1. A suggested pathophysiological pathway of how blood-brain damage in preeclampsia arise. The ischemic placenta releases harmful substances to the mother's circulation, which affect the endothelium throughout the body, including the brain endothelial cells. This impairs the blood-brain barrier with increased permeability, intracerebral edema formation, and eclampsia as a result. Reprinted by permission from Springer Nature, Current Hypertension Reports, Investigating Maternal Brain Alterations in Preeclampsia: The Need for a Multidisciplinary Effort, Bergman et al, 2019.

Cerebral autoregulation

Under normal conditions, the cerebral blood pressure is maintained stable by cerebral autoregulatory mechanisms, to ensure that the brain receives oxygen and nutrients at a constant supply. The regulatory range lies within 50-150 mmHg of mean arterial pressure and is called the static cerebral autoregulation. The static cerebral autoregulation is achieved by responsiveness in arterial myogenic tone to pressure changes, by neurogenic control and by metabolic responses to changes in CO_2 in the blood.[31,32] Once autoregulation is lost, the cerebral blood flow becomes dependent on the mean arterial pressure. Hypoperfusion (low blood flow) may cause cerebral damage due to ischemia, and hyperperfusion (high blood flow) may lead to edema or BBB disruption.[33]

In HDP, systemic inflammation and endothelial cell damage, combined with reduced smooth muscle responsiveness in the cerebral blood vessels, is assumed to disrupt cerebral autoregulation. Such changes can lead to either increased or decreased cerebral blood flow.[9] This may cause a disruption of the BBB, resulting in increased BBB permeability and fluid leakage into the brain parenchyma, which can cause cerebral edema – a condition frequently seen in eclampsia and sometimes in severe preeclampsia.[33-36] Several studies have observed that static cerebral autoregulation remains intact within the mean arterial pressure range of 50-150 mm Hg in women with preeclampsia. However, when cerebral autoregulation is impaired – characterized by decreased cerebrovascular resistance and reduced capacity of cerebral blood vessels to respond to changes in blood flow – it seems to correlate with disease severity, cerebral edema formation, and development of neurological symptoms.[33]

Static autoregulation measures the steady-state relationship between cerebral blood flow and the mean arterial pressure, and is mainly used in animal studies.[37] In contrast, dynamic autoregulation measurement is more commonly used in human studies and measures cerebral blood flow in relation to rapid changes in mean arterial pressure, as well as the time it takes to recover to baseline, resulting in an autoregulation index.

In pregnant women, cerebral blood flow variations are typically measured in response to spontaneous fluctuations in arterial blood pressure. In contrast, in other populations, these variations are often assessed by inducing blood pressure changes through methods such as postural adjustments, cuff inflation or drug administration.[38] The autoregulation index quantifies how effectively cerebral blood flow responds to changes in blood pressure. On a scale from 0 to 9, a higher autoregulation index indicates better cerebral autoregulation, with 0 representing absent autoregulation and 9 the best possible.

A study including 20 women with preeclampsia and 20 normotensive pregnant controls found that women with preeclampsia exhibited a significantly lower autoregulation index compared to the control group.[38] The dynamic cerebral autoregulation did not correlate with blood pressure measurements. Another study comparing women with eclampsia, women with preeclampsia with severe features, and women with preeclampsia without severe features to normotensive pregnant women found that the dynamic cerebral autoregulation was worse in women with eclampsia, compared with all other groups.[39]

Research suggests that the autoregulatory capacity may differ between the anterior (carotid) and posterior (vertebral) circulations. Mechanisms behind these changes are proposed to include decreased sympathetic innervation of the posterior vasculature, higher metabolic demands of the posterior parts of the brain, endothelial dysfunction, and an impaired BBB.[32]

The blood-brain barrier

The BBB is a highly restrictive semipermeable membrane of endothelial cells that prevents the circulating blood from unrestrained access to the extracellular compartment in the central nervous system. This ensures a well-adapted environment for neural function.

The BBB is formed by specialized endothelial cells, which are surrounded by a basement membrane, providing structural support to the capillaries. The basement membrane, in turn, is almost completely covered by pericytes and astrocyte end feet (Figure 2), together with the extracellular matrix these and other cells, including microglia and neurons, form the neurovascular unit.[40,41]

All of the neurovascular unit components contribute dynamically to the endothelial phenotype, by inducing expression of junctional proteins and transporters, which will regulate vascular permeability.[42] Different proteins, such as zonula occludens 1 (ZO-1), claudins and occludin, form adherence junctions and tight junctions between the endothelial cells. These ensure that there are no transcellular channels or fenestrations between the endothelial cells, thus preventing the diffusion of molecules of varying sizes; from proteins to ions. This distinguishes the BBB endothelium from endothelium in other organs, since the BBB contributes to a high transendothelial electrical resistance (TEER) and low permeability over the membrane.[26,43]

The BBB maintains a stable environment essential for optimal synaptic signaling by regulating the passage of ions and neurotransmitters. It prevents peripheral neurotransmitters, such as extracellular glutamate, from entering the brain, as its presence in the brain's interstitial fluid can cause neural damage.[40] Lining the endothelial cells on the luminal side of the BBB is the glycocalyx, which helps regulate the passage of ions and molecules between the blood and the brain.[44] Various transport proteins facilitate the influx of glucose, sodium and essential amino acids into the brain, across the BBB, while others regulate the efflux of non-essential amino acids or drugs out of the brain. Selective molecule exchange also occur via transcytosis, however, the BBB is characterized by a notably low rate of vesicular transport.[40,41] The movement of sodium changes the osmolality of plasma and may facilitate diffusion of water in and out of the brain, a physiological mechanism that is well regulated in order to protect the brain from vasogenic edema.[26]

In normal pregnancy, circulating concentrations of VEGF and PlGF are increased. These are factors that may compromise BBB integrity by increased permeability.[34] However, sFlt-1, which is also physiologically increased during normal pregnancy, is proposed to counteract the actions of VEGF and PlGF, keeping the BBB intact and preventing cerebral edema formation under normal conditions.[26] During normal pregnancy, the BBB seems to adapt and uphold its protective state.[26]

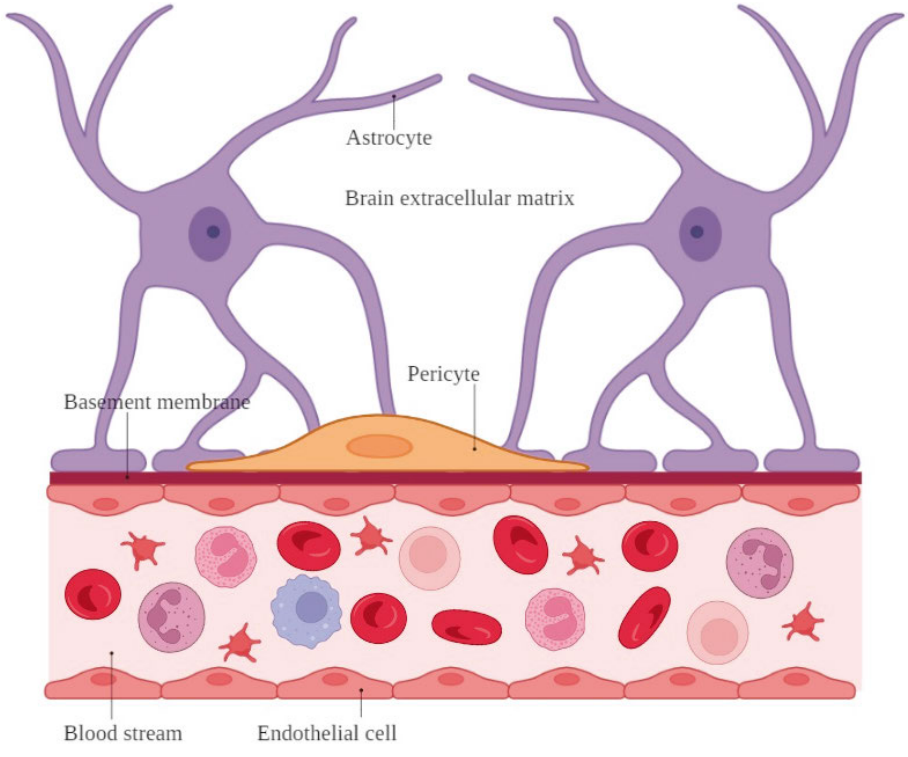

Figure 2. Schematic figure of the blood-brain barrier. Created in BioRender.com

When exposed to acutely elevated blood pressure with forced dilatation of myogenic tone, both *in vitro* and *in vivo* animal experiments have demonstrated increased BBB permeability in cerebral arteries from late-pregnant rats, compared with nonpregnant rats, in response to the same changes in hydrostatic pressure.[45]

Animal models of pregnancy, exposed to plasma from women with preeclampsia have shown increased permeability of cerebral rat veins. This effect was counteracted by co-treatment with a VEGFR2 inhibitor.[34] Several autophosphorylation sites have been described for VEGFR2. These can activate different intracellular signaling pathways.[46] Examples of these are phosphorylation of the tyrosine 951 residue (pY951-VEGFR2), which regulates endothelium permeability and cell migration; and of Y1175 (pY1175-VEGFR2), which is related to regulation of cell proliferation.[46]

Other molecules, such as tumor necrosis factor (TNF), oxidized low-density lipoproteins (oxLDL), the angiotensin II type 1 (AT1) receptor, as well as extracellular vesicles are also potential disruptors of the BBB.[47-49]

Edema formation

Impaired cerebral autoregulation, coupled with elevated circulating inflammatory and anti-angiogenic molecules, may contribute to BBB dysfunction and the cerebral edema observed in many women with eclampsia and some with preeclampsia.[9] Neuroimaging studies have demonstrated that up to 80% of women with eclampsia display vasogenic edema on MRI, in comparison to 20% of women with severe preeclampsia.[50,51] Additionally, increased diffusion, as a sign of subclinical edema, could be observed in several brain regions in the women with eclampsia.[51]

There are two main theories behind the development of cerebral edema seen in preeclampsia and eclampsia. One theory is that the high blood pressures in severe preeclampsia cause an autoregulatory breakthrough resulting in hyperperfusion and a vasogenic edema.[52] The other theory involves cerebral arterial vasospasms, possibly as an over-autoregulatory response to hypertension, which results in hypoperfusion, ischemic infarcts and a vasogenic or cytotoxic edema. Recent findings of vasospasms and hypoperfusions in women with eclampsia and vasogenic edema support the latter theory.[51]

Posterior reversible encephalopathy syndrome (PRES) is a neurological condition that can result from various underlying causes, including preeclampsia and eclampsia. PRES is characterized by vasogenic edema in the posterior parts of the brain (parieto-occipital lobes) and is often diagnosed with MRI. The clinical symptoms of PRES include headache, nausea, visual disturbances, seizures, and altered mental status.[36] The posterior cerebral circulation has less sympathetic innervation compared to the anterior circulation, making it less responsive to changes in blood pressure, such as hypertension.[10,32] This, together with a disruption of the BBB and impaired dynamic cerebral autoregulation, may contribute to the understanding of PRES development in the posterior regions of the brain even without the presence of (severe) hypertension.[10]

In animal models of preeclampsia, pregnant rats infused with sFlt-1 and sEng, as well as rats subjected to reduced uterine perfusion pressure (RUPP), developed severe preeclampsia and exhibited postpartum brain changes, including increased BBB permeability, cerebral edema, and neuroinflammation.[53,54]

Vasospasm and infarctions

An early theory, based on case reports, suggests that vasospasm of cerebral arteries contributes to the pathophysiology of cerebral ischemia and edema formation.[33,55] A case report of a woman with HELLP syndrome (hemolysis, elevated liver enzymes, low platelets) and eclampsia demonstrated that vasospasm of the cerebral arteries led to bilateral infarctions.[56]

This theory is supported by a recently published MRI angiography study, which found that 18% of women with eclampsia and 6% of women with

severe preeclampsia had vasospasm and hypoperfusion in edematous regions a few days postpartum.[51] Additionally, one-third of women with eclampsia and one out of 20 with preeclampsia exhibited cerebral infarcts.

Another MRI study of 27 women with eclampsia found that, except for vasogenic edema in 93% of women, there were also areas of cytotoxic edema and cerebral infarctions in 20% of the women. At follow-up six weeks postpartum five out of the six women had persisting signs of cerebral infarcts, even though they did not exhibit any neurological symptoms.[57]

These findings imply that cerebral infarcts may cause long-lasting scarring after eclampsia.

Methods to study the brain in preeclampsia

There are several methods to study the brain. The best suited method will depend on the research question.

Human studies

In vivo studies often pose both practical and ethical limitations, especially when it comes to the examination of pregnant women.

Neuroimaging techniques using different MRI protocols can be used to evaluate cerebral function and assess adverse outcomes *in vivo*.[58-60] These methods include intravoxel incoherent motion imaging to assess cerebral infarcts, diffusion, and perfusion, MRI angiography to evaluate vasospasms, and magnetic resonance spectroscopy to measure cerebral osmolytes and plasma osmolality. These techniques may increase the understanding of the underlying pathophysiology of cerebral complications.[51,61]

Cerebral autoregulation can be assessed *in vivo*, by non-invasively measuring changes in cerebral blood flow in response to simultaneous changes in peripheral blood pressure. This can be performed by transcranial doppler ultrasonography or MRI techniques.

Concentrations of biomarker proteins in cerebrospinal fluid (CSF) and plasma can be measured to evaluate BBB integrity.[62] Another approach is to use the CSF/serum albumin quotient (QAlb). Albumin is an endogenous protein that confines to the vascular space and is restricted from entering the brain by the BBB. Higher QAlb values have been demonstrated in women with eclampsia, and also in preeclampsia, compared with normotensive pregnant controls, indicating BBB injury.[63]

Animal models

Until recently there has been a lack of studies assessing the human BBB in preeclampsia, likely due to difficulties of studying cerebral changes in a clinical setting. Current knowledge of BBB function in pregnancy and preeclampsia is therefore largely based on animal models. Various methods can induce a preeclampsia-like state in animals, such as genetic models, surgical

induction of placental ischemia via reduced uterine perfusion pressure (RUPP), and pharmacological or dietary interventions.[64] However, since preeclampsia is a syndrome of pregnancy that only affects humans, animal studies may never truly mimic the complexity of preeclampsia.

Disruption of the BBB in preeclampsia and eclampsia has been studied in both laboratory and clinical settings, mainly through *in vivo* animal models,[9] or using *ex vivo* animal cerebral blood vessels exposed to plasma from women with preeclampsia.[34]

In animal experiments, exogenous labeled markers, such as dextran, can be used to study loss of permeability and extravasation from a leaky BBB in specific brain regions by fluorescent microscopy.[65]

In vitro systems

The aim of *in vitro* models is to mimic the *in vivo* conditions as close as possible.[66] Techniques for *in vitro* culturing of brain endothelial cells have been developed and refined since the 1970's.[67]

The simplest form of culturing is a 2D monoculture in a Petri dish or two-compartment transwell,[68] as illustrated in Figure 3.

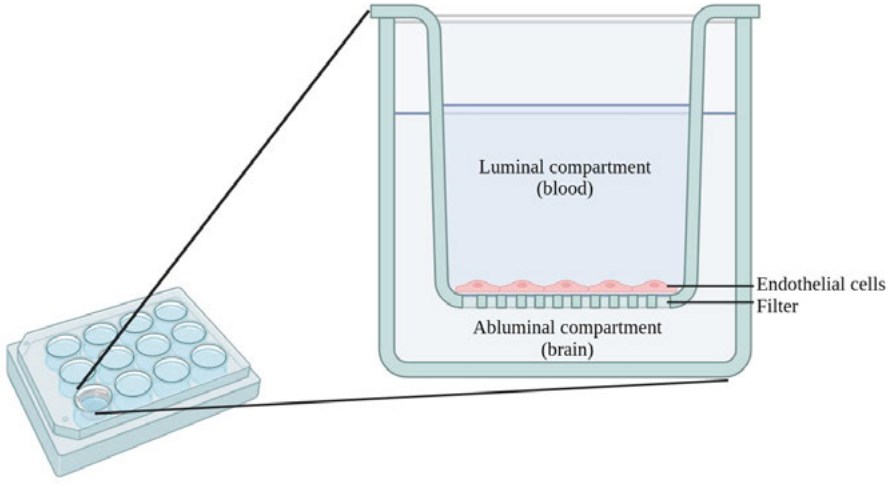

Figure 3. Schematic figure of an *in vitro* BBB model. Created in BioRender.com

Primary cultures of rodent, porcine and bovine brain capillary endothelial cells (BCEC) have been used in animal *in vitro* models.[69] These models have been valuable in understanding the physiology and pathophysiology of the BBB. Since primary cell cultures of human BCECs are not readily available, other means of studying the human BBB *in vitro* has developed. Models with human pluripotent stem cells (hPSC) differentiated into brain endothelial cells, human umbilical vein endothelial cells (HUVEC), and immortalized cell lines of human BCECs have been adopted.

Different culturing methods can be used to modify the phenotype of the BBB models. To allow for improved cell contact, even in the absence of neurovascular unit supporting cells, an extracellular matrix like collagen needs to be added to coat the membrane. Co-culturing with astrocytes or pericytes, have proven to increase TEER and decrease the permeability in *in vitro* models of both animal and human BCECs.[70]

Paper I and II of this thesis are based on an *in vitro* BBB model of human BCECs. While no *in vitro* models can accurately mimic the properties and function of the human BBB *in vivo*, they can still be useful in the understanding of physiological and pathophysiological conditions.

Cerebral biomarkers

Biomarkers of cerebral processes are often measured in blood or CSF. The measurement of cerebral biomarkers in blood can be cumbersome. The biomarkers must cross the BBB and be present in concentrations high enough for detection. The biomarkers should be specific to cerebral tissues since high expression in other organs can complicate the assessment of CNS-specific contributions. Degradation of the biomarkers by plasma proteases or clearance by liver and kidney may interfere with detection. Higher concentrations of other circulating proteins, such as albumin and immunoglobulins, in blood compared to CSF can also affect analyses.[40,71]

The cerebral biomarkers neurofilament light (NfL), tau, neuron-specific enolase (NSE) and S100 calcium-binding protein B (S100B) are all present within cells of the CNS (Figure 4).[72,73] They have been extensively studied in hypoxic and traumatic brain injury,[74] in different types of dementia,[75] and in epilepsy.[76] In preeclampsia, studies exploring these cerebral biomarkers have reported increased plasma concentrations before the onset of disease,[77,78] during disease,[79-81] and postpartum[82] in women with preeclampsia compared with normotensive pregnancies. There is, however, a lack of knowledge about the underlying pathophysiological pathways, and whether cerebral biomarkers may be useful in reflecting BBB alterations in preeclampsia.

Cerebral biomarkers could be useful for detecting early neurological involvement in pregnant women who later develop preeclampsia, as well as for staging disease severity and predicting those at risk of neurological complications once preeclampsia is diagnosed.[83] This could aid in decisions about the level or care, the initiation of neuroprotective treatment with $MgSO_4$, and the timing of delivery in cases of preterm preeclampsia.

A reliable cerebral biomarker for neurological complications in preeclampsia should originate from the CNS, and should not be found in high peripheral concentrations under normal conditions. Increased circulating biomarker concentrations would then be able to indicate either an isolated BBB injury, a BBB injury with simultaneous neuronal injury with increased secretion from

cells in the extracellular matrix, or an injured BBB as a result of previous neuronal insult.[84]

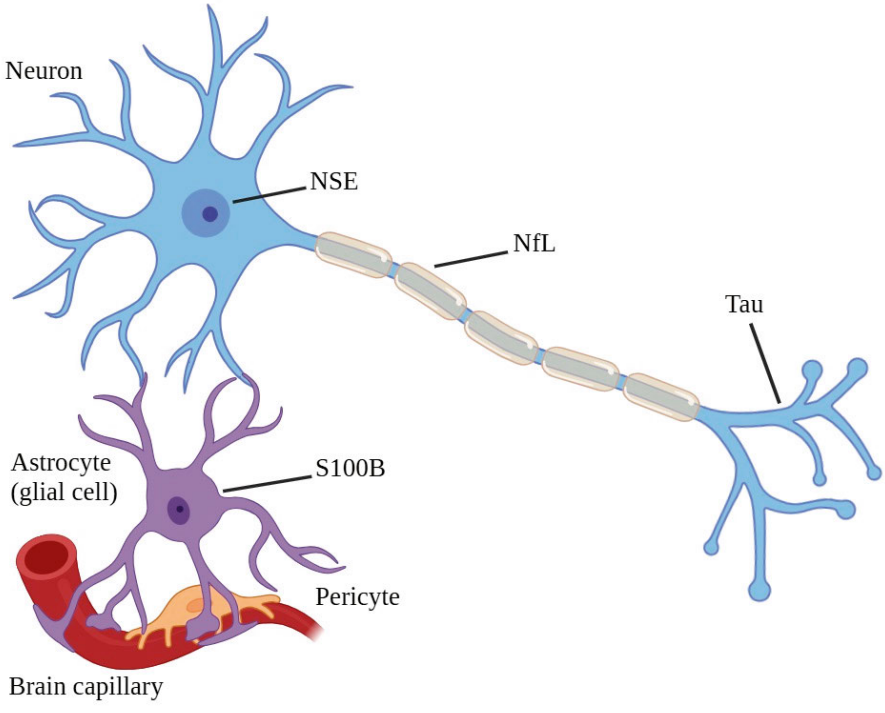

Figure 4. The origin of the cerebral biomarkers NfL, tau, NSE and S100B. Created in BioRender.com

Neurofilament light

NfL is the most abundant and soluble of three neurofilaments, with a molecular weight of 68 kDa.[85] Protein expression is predominantly found in axons, and is present in neurons of both the CNS and the peripheral nervous system.[73] Neurofilaments form a dense network along the axons of neurons, and thereby provides mechanical stability.[86]

Tau

Tau is a microtubule-stabilizing axonal protein which establishes and maintains neuronal polarity. It is localized in both neurons, astrocytes and oligodendrocytes of the brain, but is also tissue enhanced in skeletal muscle.[73] Molecular weights vary between 48-67 kDa.[87]

Neuron specific enolase

NSE is a 78 kDa glycolytic enzyme predominantly found in neurons and cells of neuronal origin.[84] NSE is also present in erythrocytes, and hemolysis of a blood sample may significantly increase NSE concentrations.[73,88]

S100B

S100B is a glial-specific protein expressed primarily by a subtype of mature astrocytes that ensheath blood vessels. Additionally, it is found in neurons, other glial cells, endothelial cells, the choroid plexus, and the ventricle walls.[73,89]

Prediction of preeclampsia

Prediction and prevention of preeclampsia poses major challenges in obstetric care. Several risk factors for developing preeclampsia have been identified, including a personal or family history of HDP, maternal age, primiparity, obesity and diabetes.[90] However, their individual predictive values are low for the general pregnant population.

Various prediction models have been developed to assess the risk of preeclampsia, primarily focusing on maternal risk factors and medical history. Many of these models incorporate various biomarkers, such as mean arterial pressure, uterine artery pulsatile index (PI), and different serum biomarkers (e.g., PlGF and PAPP-A).[91]

In Sweden, pre- and antenatal screening for preeclampsia is based on scoring of risk factors, divided into moderate- and high-risk (Table 1). Women are considered at high risk of developing preeclampsia if they have at least one high-risk factor or three moderate-risk factors.[13]

Table 1. Risk factors for preeclampsia, as stated by the Swedish Society of Obstetrics and gynecology.

Moderate-risk factors:	**High-risk factors:**
Nulliparity	Auto immune disease (SLE or APS)
Family history of preeclampsia	Previous preeclampsia or eclampsia
BMI > 30	Previous GH with delivery before 34
Age > 40 years	weeks, IUGR, IUFD or ablatio
Pregnancy interval > 10 years	Diabetes type 1 or 2
SBP > 130 or DBP > 80 mmHg at admission	Multiparity
African heritage	Chronic kidney disease
Verified obstructive sleep-apnea	Chronic hypertension
”White coat hypertension”	*In vitro* fertilization with oocyte donation
Previous GH	

Abbreviations: APS, anti-phospholipid syndrome; BMI, body mass index; DBP, diastolic blood pressure; GH, gestational hypertension; IUFD, intrauterine fetal death; IUGR, intrauterine growth restriction; SBP, systolic blood pressure; SLE, systemic lupus erythematosus

Since the 1970's, many studies have evaluated the effect of aspirin for primary prevention of preeclampsia and the safety of aspirin use during pregnancy.[92] There is evidence that the use of aspirin, initiated before 20 weeks of gestation and taken until 36 weeks, reduce both incidence of preterm preeclampsia and the need for neonatal intensive care in a high-risk population.[93,94] At the time of writing this thesis, aspirin prophylaxis is recommended to Swedish women with any high-risk factor of preeclampsia, or three or more moderate-risk factors, according to Table 1.[13]

Prediction of cerebral complications

Predicting cerebral complications of preeclampsia in women who are already diagnosed with the disease is challenging as there is currently no test or symptom that has been proven to be reliable. Yet, it would be desirable to identify a cerebral biomarker or a model for predicting cerebral outcomes, which could aid clinicians in decisions about optimal care for the mother and with interventions such as $MgSO_4$ treatment and timing of delivery.

In a 2024 systematic review and meta-analysis encompassing 110 studies and 1,500 predictive performance tests for various maternal and perinatal outcomes, no test demonstrated good predictive performance for eclampsia. The predictors evaluated for eclampsia included aspartate aminotransferase (AST) > 2 standard deviations (SD) in one test and vivid deep tendon reflexes in another. Due to the low incidence of eclampsia and other neurological complications in the included studies, meta-analyses could not be conducted for these outcomes.[95] Another systematic review focused solely on eclampsia as the outcome included 11 studies but did not identify any predictors for diagnosing or ruling out eclampsia.[96] Neurological adverse outcomes of preeclampsia are rare in high-income countries, which often keep the largest registries or cohorts of women with preeclampsia. The absence of standardized definitions for exposures and outcomes in studies hampers the ability to compare results and aggregate data effectively.

Clinical presentation

Preeclampsia can affect various organ systems, as they are all susceptible to the inflammation and endothelial injury resulting from its altered angiogenic profile.[1] Oliguria and increased serum creatinine are signs of acute kidney failure. Liver dysfunction may cause epigastric pain or right upper quadrant pain, and it may cause severe complications like subcapsular liver hematoma and rupture. A feared complication of preeclampsia is HELLP syndrome, in which both the liver and the coagulation system is affected, with risk of hemorrhage and microvascular thrombosis, leading to disseminated intravascular

coagulation. Symptoms from the cardiovascular system may include heart failure and pulmonary edema.

Clinically, it seems that early- and late-onset preeclampsia differ in severity of disease. Early-onset preeclampsia is more often associated with placental insufficiency resulting in IUGR and the need for preterm delivery, as well as higher risk of short- and long-term maternal morbidity.[1,97] Even though late-onset preeclampsia is not as often complicated by IUGR as early-onset disease, it contributes to a large portion of maternal morbidity and severe complications, due to the increasing incidence of preeclampsia with advanced gestational age.[16,23,98]

Acute cerebral complications

Neurological symptoms of preeclampsia include persisting visual disturbances (blurred vision, scotomas and temporary blindness), hyperreflexia with foot clonus, severe headache and altered mental status.

Sometimes these symptoms precede eclampsia, however, eclampsia may also occur without any warning signs, sometimes presenting as the first manifestation of preeclampsia without preceding hypertension and proteinuria.[96]

Women experiencing eclampsia or severe preeclampsia may present with neurological symptoms such as persistent severe headaches, visual disturbances, and altered mental status. Neuroimaging in these cases frequently reveals edema in the parietal and occipital lobes, indicative of PRES. Unlike stroke, PRES has been assumed to be reversible if diagnosed and managed in time.[36,52]

While stroke is a rare complication of preeclampsia, those affected are at a higher risk for hemorrhagic stroke compared to the general population, where ischemic stroke predominates. Hemorrhagic strokes tend to be more fatal than ischemic strokes.[29,99] Cerebral hemorrhage or severe PRES with increased intracranial pressure may, in the worst-case scenario, lead to brain herniation.

Management

Once preeclampsia is diagnosed the mother needs to be assessed. If she has any severe end-organ involvement, such as eclampsia, uncontrolled blood pressure, HELLP syndrome, or pulmonary edema, delivery is needed irrespective of the gestational age. The mother needs to be stabilized, and then birth is planned within 12-48 hours,[13] preferably allowing administration of prophylactic magnesium and/or corticosteroids for the neonate's neuroprotection and pulmonary maturation, if preterm and the fetus is considered viable.

If the mother is relatively stable and the pregnancy is preterm one may consider expectant management to prolong pregnancy for the maturation of the fetus. In these cases, obstetricians are faced with the dilemma of timing of

delivery. Prolonging pregnancy carries a risk of worsening of the disease and development of severe complications for both mother and neonate. Early delivery is associated with an increased morbidity and mortality of the preterm neonate.[16]

With term preeclampsia, the ethical and medical dilemma of inducing labor is not as difficult, since there is not much to gain with continuing the pregnancy. However, term preeclampsia accounts for the majority of cases and thus contributes to the larger part of maternal mortality and morbidity.

Antihypertensive treatment is recommended to avoid acute severe hypertension. While guidelines may vary slightly worldwide in recommendations of when to initiate antihypertensive treatment, which drugs to use, and at what levels blood pressure should be maintained at, there is consensus that urgent treatment is required at SBP ≥ 160 mmHg and/or DBP ≥ 110 mmHg.[11]

Once a decision has been made to initiate labor, delivery can be achieved either by inducing vaginal labor or by performing a cesarean section. The only known curative treatment for preeclampsia is delivery of the placenta.[20] Despite this, maternal risks do not immediately resolve postpartum; difficulties in treating blood pressure and the risk of eclamptic seizures may persist for days or even weeks. Therefore, postpartum monitoring of blood pressure and signs and symptoms is of utmost importance.[11,100]

Management of cerebral complications

At SBP ≥ 160 mmHg and/or DBP ≥ 110 mmHg blood pressure management is urgently recommended, as these levels are associated with increased risk of stroke/cerebral hemorrhage.[11]

$MgSO_4$ is the only known drug that prevents eclamptic seizures, reducing the risk by half.[11,18] Studies of animal models, and one *in vitro* study using human brain endothelial cells, have found evidence of improved BBB function by reduced BBB permeability, reduced neuroinflammation and reduced cerebral edema after $MgSO_4$ administration.[101-104]

Currently, no neuroprotective treatment is available for the prevention of neurological symptoms or sequalae from preeclampsia and eclampsia.

In their 2014 guidelines, the ISSHP recommended that, due to cost-benefit reasons, all women with preeclampsia in low- and middle-income countries should receive $MgSO_4$ treatment, whereas only women with severe preeclampsia in high-income settings should receive treatment.[11] This is, however, not a feasible recommendation since it would incur substantial costs in resource-limited settings. This is also where the incidence of preeclampsia is the highest. Moreover, the administration of $MgSO_4$ is recommended for 24h after delivery, and increased monitoring is required due to the risk of toxicity of the treatment.

Maternal morbidity

Beyond maternal mortality rates, it has been estimated that for every maternal death there are 20-100 women suffering from complications or morbidity following HDP.[1,105,106] Maternal morbidity is defined by the World Health Organization (WHO)[107] as:

> "Any health condition attributed to and/or complicating pregnancy and childbirth that has a negative impact on the woman's wellbeing or functioning."

Several meta-analyses and systematic reviews have concluded that women who have experienced preeclampsia face an increased risk of developing cardiovascular diseases later in life. This risk may be higher for women who experienced preterm preeclampsia compared to those with term preeclampsia, however, the findings are not entirely consistent.[5,97,108,109] A Swedish register-based study found a stronger association with severe preeclampsia in the first pregnancy and later development of ischemic heart disease, compared with preeclampsia without severe features or gestational hypertension. Moreover, recurrent HDP was more closely associated with ischemic heart disease than non-recurrent HDP.[110] Subsequently, a Danish register-based study found a strong association between women with HDP and later development of chronic hypertension and type 2 diabetes mellitus.[111] A more recent systematic review and meta-analysis, containing 22 studies and > 6.4 million women, has reinforced these findings. They found preeclampsia to be associated with a fourfold increased risk of heart failure and a twofold increased risk of coronary heart disease and stroke.[5]

Neuroradiological findings

Traditional CT and MR imaging protocols may not be sufficient in revealing more subtle signs of cerebral and BBB injury. However, some imaging techniques are not suitable for use during pregnancy because they may pose potential safety concerns. Gadolinium-based contrast agents should be used cautiously during pregnancy due to potential risks to the fetus. Additionally, MRI scans with magnetic fields stronger than 3 Tesla are not yet standard practice due to limited knowledge about how stronger fields affect the fetus.

Several years after pregnancy, women who had experienced preeclampsia exhibited increased BBB leakage compared to those with normotensive pregnancies. This leakage was more prominent in the white matter of the frontal, temporal, and occipital regions, as well as in the grey matter in the frontal and parietal regions. These findings were detected using advanced 7 Tesla MRI technology, which enables the identification of more subtle cerebral changes that might go unnoticed with standard MRI methods.[58] These widespread signs of BBB impairment may indicate persistent endothelial dysfunction,

potentially explaining the increased risk of cerebrovascular diseases observed in women with preeclampsia later in life. The cerebral changes noted in women after experiencing preeclampsia and eclampsia may be lasting effects of a previous episode of PRES. This suggests that PRES may not be entirely reversible.

Many neurological disorders affecting women in later life, such as vascular dementia, stroke, and cognitive decline, are caused by cerebral small vessel disease. On magnetic resonance imaging, cerebral small vessel disease can manifest as changes in white matter, brain atrophy, micro bleeds, lacunar infarctions and enlarged perivascular spaces.[112] Similar findings have been observed in women after preeclampsia. On MRI scans several years after an index pregnancy with preeclampsia, compared with parous controls, an increase in cerebral white matter lesions could be demonstrated.[113] This supports the theory that preeclampsia and cardiovascular and cerebrovascular diseases share similar pathophysiological mechanisms.[114] Other MRI brain studies examining women 5-15 years postpartum, found that women with previous HDP or preeclampsia demonstrated smaller brain volumes, reduction in cortical gray matter volume, more pronounced white matter changes in the temporal lobes, as well as altered microstructural integrity, when compared with women with normotensive pregnancies.[115,116] White matter microstructural changes correlated positively to time after index pregnancy, indicating that cerebral alterations instigated by preeclampsia may well advance over time.[116] Women who experienced preeclampsia and late-life hypertension have also been demonstrated to have smaller grey matter volumes compared to those who had preeclampsia without later hypertension and those with normotensive pregnancies, regardless of their hypertension status in later life.[117]

Medium-term neurological disorders

Little is known about possible associations between HDP and neurological disorders in the months to years following childbirth. A retrospective register-based study from Canada has reported an association between eclampsia and future seizure disorder with an aHR of 5.4 (95% CI 2.4–12.1), and between preeclampsia and future seizure disorder (aHR 2.0, 95% CI 1.2–3.2), in reference to normotensive pregnancy. Their mean follow-up time was 7-9 years after the index pregnancy.[118]

A study involving interviews with over 100 women who experienced eclampsia during their index pregnancy found that approximately 50% reported one or more persistent subjective symptoms up to two years postpartum. Approximately 20% of the women reported experiencing migraines and headaches, while 14% reported symptoms of depression.[8] Beyond relying on self-reported symptoms, this study lacked a control group, limiting the interpretation of results. At the time of writing this thesis, no other studies mentioning

migraine, headache, sleeping disorders or mental fatigue in association with HDP, were found.

Cognitive decline

The risk of cognitive decline increases with age. For women, this risk may also be influenced by hormonal changes and a history of HDP.[119]

A 2017 systematic review and meta-analysis found no evidence of cognitive impairment on standard neurological tests following preeclampsia. However, this analysis excluded women who had experienced eclampsia, potentially omitting the most severe cases and limiting the study's ability to detect associations.[120] In 2024, another systematic review and meta-analysis investigated the association between HDP and cognitive function. The study found that women with a history of HDP exhibited poorer cognitive performance in memory, attention, and executive function domains.[6] However, the authors noted the wide variety of cognitive tests used across studies, which complicates analysis and interpretation.

In a study, women with HDP, particularly those who had experienced eclampsia, scored lower on questionnaires assessing cognitive failure compared to normotensive controls.[121] Women with previous eclampsia scored higher in a Cognitive Failures Questionnaire years after the index pregnancy compared to healthy parous controls in another study.[122] Women with HDP have also been found to perform worse on processing speed tests decades postpartum.[115]

A study involving women around 60 years old, approximately 35 years after their index pregnancy, conducted comprehensive cognitive evaluations by neuropsychologists and neurologists. The findings indicated a trend toward increased cognitive impairment in women with a history of preeclampsia compared to women with normotensive pregnancies, however, these results were not statistically significant.[123] Since then, a study measuring cognitive performance in women 15 years after experiencing HDP, as compared to normotensive pregnancy, has been published. Various cognitive domains were evaluated, confirming an association between HDP and poorer working memory and verbal learning.[124]

Dementia

Several studies have shown an increased risk of developing vascular dementia later in life after a pregnancy complicated by preeclampsia. This was supported by a 2024 systematic review and meta-analysis, analyzing women with HDP and outcomes of dementia and cognitive decline. Preeclampsia was associated with an increased risk of vascular dementia with an aHR of 1.89 (95% CI 1.47-2.43), compared to normotensive women. The association between preeclampsia and Alzheimer's dementia, as well as any dementia, was not as clear. However, in women over 65 years of age, preeclampsia was associated

with an increased risk of both Alzheimer's (aHR 1.92, 95% CI 1.35-2.73) and any dementia (aHR 1.87, 95% CI 1.21-2.91).[6] Two nationwide register-based studies from Sweden and Denmark, each involving over 1.1 million women, reported that those with a history of preeclampsia had a 1.6-3.4 times higher risk of developing vascular dementia compared with parous women without a history of preeclampsia.[125,126]

When looking at all-cause mortality after HDP, women with previous HDP had a high excess mortality risk of dying from Alzheimer's disease (aHR 3.4, 95% CI 1.0-11.8).[127]

Cerebrovascular disease and stroke

The increased risk of vascular disease following preeclampsia also includes cerebrovascular disease and stroke. A systematic review and meta-analysis from 2008, including five case-control studies and ten cohort studies, concluded that women with a history of preeclampsia or eclampsia had a doubled risk of cerebrovascular disease.[108] A more recent systematic review and meta-analysis from 2023 analyzed the risk of stroke after HDP. The majority of included studies focused on preeclampsia, which had an adjusted risk ratio (aRR) of 1.75 (95% CI 1.56-1.97) for any stroke, and similar risk for ischemic stroke (aRR 1.74, 95% CI 1.46-2.06). Studies on hemorrhagic stroke (aRR 2.77, 95% CI 2.04-3.75) exhibited a higher degree of heterogeneity, suggesting that these results should be interpreted cautiously. The number of studies on gestational hypertension and later stroke were fewer, however, an increased risk of any stroke (aRR 1.23, 95% CI 1.20-1.26) and ischemic stroke was supported. The follow-up time of the included studies varied considerably, from just a couple of years to several decades.[7]

An increased risk of mortality due to stroke in women with hypertensive disorders of pregnancy has been reported.[127]

Psychosocial functioning and quality of life

Several studies have highlighted the need for follow-up of psychosocial parameters in women with previous preeclampsia, and especially after severe preeclampsia. A short-term follow-up study at 6 weeks postpartum on health-related quality of life (QoL) found that women with preeclampsia with severe features reported lower on all items than women with preeclampsia without severe features. At 12 weeks, overall QoL improved in both groups, however, the mental health, role emotional and social functioning still remained poorer in the group of women with severe preeclampsia.[128] Admission to neonatal care and perinatal death were contributing factors. Preterm birth and adverse neonatal outcomes have also been reported by others to affect the psychosocial well-being of women after severe preeclampsia.[129,130]

A study found that women with preeclampsia reported worse mental QoL, compared with normotensive controls two years after pregnancy. Women with severe preeclampsia had worse mental QoL than women with preeclampsia without severe features.[131] In an interview-based follow-up study of women after eclampsia, many women reported persistent symptoms consistent with post-traumatic stress disorder, such as problems to concentrate or recalling (22%), tiredness (9%), and restlessness (7%) two years postpartum.[8]

Another study reported that women with previous preeclampsia scored worse on QoL and social functioning, on average four years after their first birth, compared with normotensive controls.[121] Women with eclampsia demonstrated notably poorer outcomes. No differences were observed when comparing women with early-onset versus late-onset preeclampsia, suggesting that preterm birth and associated complications related to caring for a preterm infant did not mediate these findings. This contrasts with previous findings. Posttraumatic stress symptoms appeared to partly explain the association between preeclampsia and long-term health effects. However, the significant impact of preeclampsia persisted even after accounting for these symptoms, indicating that psychological trauma from experiencing severe pregnancy complications alone may not fully explain these long-term effects.[121] This is consistent with findings from another study, in which women with early-onset severe preeclampsia were compared with normotensive women delivering preterm. On average, seven years postpartum, most women in both the case and control groups exhibited high levels of posttraumatic stress symptoms, however, women with a history of preeclampsia had higher scores. The groups scored the same on depression scales.[132] A study on women with previous preeclampsia, showed that high resilience seemed to lower the risk of depression in the years following pregnancy, and also promoted higher mental QoL, when compared with women with preeclampsia and low resilience.[133]

Sick leave

Sick leave during pregnancy has been studied from many aspects. However, publications on changes in sick leave patterns before and after pregnancy are scarcer. The few studies comparing changes in sick leave before and after pregnancy have primarily focused on effects of parity and different occupational categories.[134-137] The Swedish Social Insurance Agency published a report in 2014, stating that psychiatric disorders were the predominant reason for sick leave prior to women's first pregnancies. In the seven years following the first childbirth, however, pregnancy-related complications, primarily involving musculoskeletal disorders, accounted for a larger proportion (40%) of sick leave.[138]

Two Swedish studies were found to have reported on sick leave after pregnancies complicated by intercurrent disease. One recent cohort study assessed sick leave following pregnancy in women with intercurrent systemic lupus

erythematosus (SLE) compared with healthy pregnant controls. Not surprisingly, women with SLE, a chronic autoimmune disease, had a higher prevalence of sick leave.[139] The other study was a retrospective questionnaire study comparing QoL, dietary intake, and physical activity in women four years after an index pregnancy with or without gestational diabetes. Women with gestational diabetes reported a higher level of sick leave (exceeding one week) than controls, however, the study could be prone to recall bias and results should be interpreted with care.[140] Only one study was found that reported the incidence of postpartum sick leave following severe preeclampsia. This Dutch study indicated that 72% of the included women had resumed work at one year postpartum, whereas 9% remained on sick leave. However, the study lacked a control group for comparison.[129]

Aims of the thesis

The overall aim of this thesis was to gain knowledge of how preeclampsia affects the blood-brain barrier and whether circulating cerebral biomarkers correlate to blood-brain barrier impairment. Additionally, to investigate how preeclampsia and eclampsia influence women's neurological health and sick leave in the years following childbirth.

The specific aims of the studies were:

I To study the effect of plasma from women with preeclampsia in a human *in vitro* blood-brain barrier model.

II To correlate plasma concentrations of cerebral biomarkers in women with preeclampsia with transendothelial electrical resistance over the blood-brain barrier in a human *in vitro* model.

III To investigate the risk of new-onset medium-term neurological disorders after pregnancies complicated by gestational hypertension, preeclampsia, or eclampsia.

IV To examine sick leave patterns during the second year postpartum in women who experienced preeclampsia.

Material and methods

Overview of the studies

Paper	Study design	Study population	Period	Exposure	Outcome
I	Experimental *in vitro* study	28 women with preeclampsia, 28 normotensive pregnant women, and 16 non-pregnant women, recruited in Uppsala, Sweden	2013-2016	Preeclampsia	Changes in TEER, permeability and VEGFR2 expression in the *in vitro* model
II	Experimental *in vitro* study	28 women with preeclampsia, 28 normotensive pregnant women, and 16 non-pregnant women, recruited in Uppsala, Sweden	2013-2016	Preeclampsia	Cerebral biomarker plasma concentrations in correlation with TEER in the *in vitro* model
III	Register-based cohort study	648,385 primiparous women in Sweden	2005-2019	Gestational hypertension, preeclampsia, eclampsia	A composite of neurological disorders
IV	Register-based cohort study	95,716 primiparous women in Sweden	2016-2019	Preeclampsia	Number of sick leave days exceeding 14 days in the second year postpartum

Ethical considerations

Paper I and II

Ethical approval for Paper I and II was obtained from the Uppsala Ethical Review Board in 2012 (approval number 2012/087) regarding collection and analysis of plasma samples from the women. In 2017, a supplementary ethics application was approved (approval number 2012/087/1) to permit the export of plasma samples to Chile for analysis in the *in vitro* BBB model. Written informed consent was originally obtained from all participating women. The women underwent blood sampling at enrolment in the study, which might have caused mild harm, other than that the women or their fetuses would not have suffered any physical harm.

The primary isolate for the hCMEC/D3 cell line was donated from a patient for research purposes in 2005, and has been extensively used ever since.[141]

Paper III and IV

The ethics application covering Paper III and IV was approved from the Uppsala Ethical Review Board in 2019 (approval number 2019-04925). The dataset was pseudonymized using a unique serial number upon delivery. Register-based studies using pseudonymized data are exempt from consent requirements according to Swedish data protection legislation.

Laboratory methods

The Human Cerebral Microvascular Endothelial Cell line

The Human Cerebral Microvascular Endothelial Cell line (hCMEC/D3) was derived from BCECs from a woman undergoing surgery for control of epilepsy in 2005.[141] Micro vessels were isolated from tissue excised from the temporal lobe, and cells were immortalized, cloned and characterized for brain endothelial phenotype.[66]

hCMEC/D3 cells demonstrate BBB characteristics such as the formation of tight junctions and the capacity to exclude plasma components such as drugs. In culture, these cells functionally express important active transport proteins. This cell line has been extensively used to test BBB function, including pathological and drug transport mechanisms. It has also been instrumental in understanding the brain endothelium's response to various human pathogens and inflammatory stimuli.[66,142,143]

The model illustrated in Figure 3 is a 2D *in vitro* model where monolayers of endothelial cells are grown on the upper surface of a semipermeable filter in a two-compartment cell culture system (transwell), separating the blood (apical chamber/luminal compartment) from the brain (basolateral chamber/abluminal compartment). Different media can be added to the model to study their effect on the BBB.

BBB integrity can be estimated with different methods *in vitro*:

- To evaluate the permeability of the BBB, stained tracer molecules, such as fluorescently labeled dextrans, can be added to the apical chamber. These are large molecules that does not normally pass the BBB. Subsequently, the amount of molecules that pass the barrier into the basolateral chamber are measured.[65,143]
- The barrier permeability can also be assessed through measurement of the TEER. The electrical resistance over the BBB is normally high and alterations in resistance over the basement membrane can result in loss of BBB integrity and an increase in BBB permeability.[143] Electrodes can be placed in each of the two transwell compartments for measurement of the electrical resistance over the barrier.[69]
- By measuring different proteins, the function of the BBB tight junctions can be evaluated, as the loss of a protein may cause leakage between the cells. Transport proteins are of interest both in understanding the pathological formation of intracerebral edema and how different drugs affect the brain under normal conditions, as well as when the BBB function is impaired. The expression of proteins can be measured by PCR or immunodetection techniques, including Western blotting and immunocytochemistry.[69]

The use of a human *in vitro* model of the BBB, as opposed to animal-based models or the use of human plasma in animal models, might possibly reflect the human BBB to a greater extent.

The hCMEC/D3 *in vitro* model

The hCMEC/D3 cell line was used for the *in vitro* experiments in Paper I and II.[66] Monolayers of cells were seeded at a density of 20,000 cells/well on semipermeable plates, pre-coated with collagen. A growth supplement was used as a culture medium, and cells were incubated at 37°C, 5% CO_2. Once cells had reached 100% confluence, they were ready for experiments. Six hours prior to experiments the culture medium was replaced by a basal medium without growth supplements. The cultured cells were exposed to thawed plasma from women with preeclampsia, women with normotensive pregnancies, or non-pregnant women for 12 hours.

All analyses were carried out in duplicates by researchers blinded to groups.

Measurement of transendothelial electrical resistance

For TEER experiments in Paper I and II, an epithelial Volt/Ohm meter with two “chopstick” electrodes was used for measurements. One electrode was placed in each compartment to measure the TEER (Figure 5). Transwells with cells demonstrating electrical resistance below 20 Ωcm^2 prior to plasma exposure were excluded. Measurements were performed both before adding

plasma (baseline), and after 12h incubation with plasma. To estimate the effect of plasma on BBB permeability, the ΔTEER value was calculated as the arithmetic difference between baseline TEER values and those after plasma exposure, expressed as a decrease from baseline.

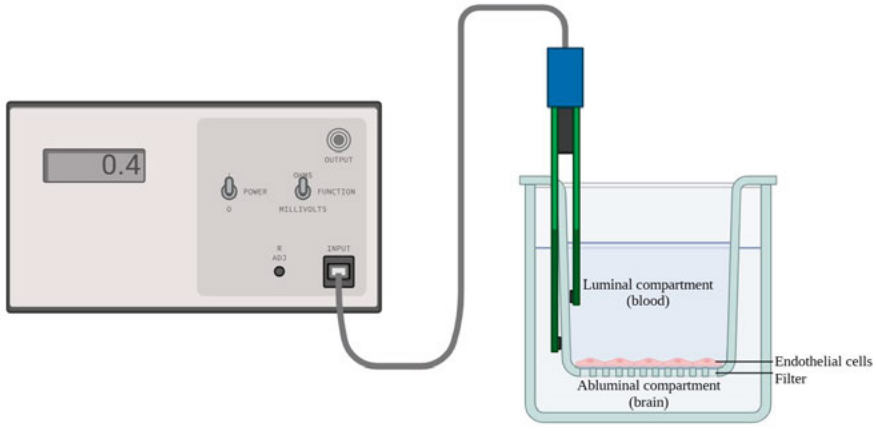

Figure 5. TEER measurement with an EVOM2 Voltmeter, electrodes in each compartment of the transwell. Created in BioRender.com

Measurement of permeability to FITC-Dextran 70 kDa

For permeability experiments in Paper I, cells in the apical chamber were incubated with a solution of 1 μM fluorescein isothiocyanate (FITC)-Dextran 70 kDa for one hour. Subsequently, medium was collected from the basolateral chamber and stored in amber tubes at −20°C. The fluorescence in the collected media was measured with filters at 485 and 540 nm. For calculations, the background values from transwells without any cells or collagen coating were subtracted from each measurement.

Quantitative PCR for measuring mRNA levels of VEGFR2

After the cells had been exposed to plasma, mRNA levels of VEGFR2 were quantified by quantitative polymerase chain reaction (qPCR).

Measurement of cerebral biomarkers

Most assays of cerebral biomarkers are immunochemical; i.e., they utilize antibodies to quantify a substance in a sample.

The enzyme-linked immunosorbent assay (ELISA) technique uses a capture-antibody to bind a certain biomarker, its antigen. A secondary detection-antibody is added, which binds to the antibody-antigen-complex. After a washing procedure, an enzyme is added which generates a signal that can be detected and translated into a concentration.[144] To test the reliability of the immunoassays results, intra- and inter-assay coefficients of variability (CV)

should be assessed. An intra-assay < 10% CV and an inter-assay < 15% CV is generally considered acceptable.

For Paper II, plasma NSE and S100B concentrations were measured with ELISA using commercially available kits, and samples were run according to the manufacturer's recommendation. Intra- and inter-assay CVs were consistent with reliable measurements of the immunoassay.

The single molecule array (Simoa) technology uses the same principle as the ELISA**,** but enables a single-molecule read-out, which allows for detection of biomarkers at ultra-low concentrations.[74]

For Paper II, plasma NfL concentrations were measured using an in-house Simoa method, and tau concentrations were measured with the Human Total Tau 2.0 kit and the Simoa platform. Measurements were performed in one round of experiments, using one batch of reagents by laboratory technicians who were blinded to clinical data. Two quality control samples were run in duplicates in the beginning and the end of each run, with CVs indicative of reliable results for both NfL and tau.

Social insurance in Sweden

The Swedish social insurance system is extensive and offers financial security for Swedish residents. It includes health and sickness benefits, parental and family benefits, work and unemployment benefits, pension and elderly support, as well as disability and support benefits. It is primarily administered by the Swedish Social Insurance Agency.[145] Social insurance data is registered in the Swedish Longitudinal Integrated Database for Health Insurance and Labor Market Studies (LISA database).[146]

Health insurance

The Swedish health insurance covers all who lives and works in Sweden, and provides an income for those who, due to illness or injury, are temporarily unable to work. Sickness benefit may be granted for 25%, 50%, 75%, or 100% reduced work capacity.

During the first 14 days of sick leave, the employer is required to pay sick pay, except for the first day which is a qualifying day. If the work capacity remains reduced after 14 days, sickness benefit is provided by the Social Insurance Agency.

Sick leave data is reported to the LISA database by calendar year.

Parental insurance

In Sweden, parents are entitled to 480 days of paid parental leave per child, which is shared between the parents, often with the mother taking a slightly larger share of the leave. It is not uncommon that a woman stays at home for one year after the birth of the first infant.[147]

National Registers

The Swedish National Board of Health and Welfare and Statistics Sweden hold several registers that are extensively used as data sources for statistics and medical research. It is mandatory to report to these registers and no consent is needed from individuals. After ethical approval, pseudonymized data can be available for research.

For this thesis, data from the National Medical Birth Register (MBR), the National Patient Register, the Cause of Death Register, and the LISA database was provided by The Swedish National Board of Health and Welfare. Statistics Sweden gave access to information from the Total Population Register and the Education Register.

Linkage of registers

Each individual living in Sweden is assigned a unique personal identification number upon birth or immigration. This facilitates the linkage of data across national registers for epidemiological and medical research purposes.[148]

The data used in Paper III and IV for this thesis was extracted from the registers in 2021. Through the personal identification numbers all relevant data could be collected, and linkage between registers was performed by the Swedish Board of Health and Welfare. Data regarded all births in Sweden from January 1, 2005 to December 31, 2018, with data on follow-up parameters until December 31, 2019. The dataset was pseudonymized using a unique serial number upon delivery.

Medical Birth Register (MBR)

The MBR holds information on 98% of all pregnancies and deliveries in Sweden. Data covers both mothers and their newborns, and has been registered since 1973.[149] The high coverage is made possible by the fact that antenatal and maternity care is free of charge in Sweden, home deliveries are rare, and information from the medical records is automatically transferred to the register. Registration starts at the first antenatal visit and information is collected prospectively throughout pregnancy, delivery and the neonatal period using standardized records. Data is transferred to the MBR after delivery.

Data regarding the mother, such as age, smoking habits, parity, and pregestational diagnoses (recorded by midwives filling in checkboxes at the first antenatal visit) can be found in this register. It also contains data on maternal diagnoses, gestational age at delivery, and other delivery characteristics. Diagnoses and procedure codes covering complications during pregnancy and the perinatal period are classified according to the Swedish version of the International Classification of Diseases, 10th revision (ICD-10) at discharge from the hospital. The MBR also provides data on infants, such as birth weight, gestational age at birth, single/multiple birth and stillbirth.[149,150]

National Patient Register

The National Patient Register contains nationwide information regarding specialized in- and outpatient care since 1987 and 2001, respectively. It includes information on ICD-10-coded diagnoses and dates of hospital admissions, discharges, and out-patient visits. The National Patient Register does not cover data on diagnoses or visits in primary health care.[151]

Cause of Death Register

The Cause of Death Register comprises data on when death occurred and the cause of death for all individuals registered in Sweden since 1952.[152]

Total Population Register

The Total Population Register contains information about the Swedish population (e.g., civil status, country of birth, and migration) based on data from the Swedish Tax Agency's population register since 1968. The quality of data in the register is generally regarded as high. Most data on births, deaths, country of birth, and civil status are reported by professional and administrative personnel and information is transferred on a daily basis. However, data on migration may be the cause of both under- (immigration) and over coverage (emigration).[153,154]

Education Register

The Education Register holds longitudinal data on the highest level of formal education (university, upper secondary school degree, or < 12 years of school attendance) and educational orientation of the population of Sweden. The register is annually updated since 1985.[155]

The Longitudinal Integrated Database for Health Insurance and Labor Market Studies (LISA)

The LISA database was formed in 2003 but covers data back to 1990. Information is annually retrieved from the Swedish Social Insurance Agency and covers all persons aged 16 or older who are registered in Sweden. The LISA database provides a unique opportunity to study the impact of disease on working capacity.

The LISA database includes detailed information on health insurance, parental insurance, and unemployment insurance at an individual level. This enables the study of individuals' transition over time between, for instance, gainful employment, unemployment, and illness. LISA only covers sick leave episodes exceeding 14 days, containing data on gross and net days of sick leave, and incomes from social insurances. However, the diagnoses associated with the period of sick leave is not reported. LISA also contains data on ICD-coded causes of death, dates of death, and demographic information such as migration status, linked through the Total Population Register.[146,156]

Study populations and study design

Paper I and II

Paper I and II were experimental studies aiming att investigating possible impairment of the BBB in a human *in vitro* model.

Study participants were recruited from the obstetric ward or the outpatient clinic at Uppsala University Hospital, Sweden, as part of a cross-sectional Magnetic Resonance Spectroscopy study of women with preeclampsia during 2013-2016.[157]

Women with singleton pregnancies and gestational ages between 22 weeks and 0 days (22+0) and 41 weeks and 6 days (41+6) were included. Exclusion criteria included pregestational chronic hypertension, pregestational or gestational diabetes mellitus, chronic kidney disease, and contraindications for MRI, such as claustrophobia or the presence of a pacemaker.

Two control groups were recruited. The normotensive pregnant group was recruited through informational posters at antenatal outpatient clinics in Uppsala and at university facilities. The non-pregnant control group was recruited through Facebook and local networks.

All participants underwent blood pressure measurement and blood sampling within 12 hours after enrolment in the study. Manual systolic and diastolic blood pressures were measured in supine position in the right arm after a 15-minute rest. Plasma samples were collected into Vacutainer tubes containing lithium heparin, then centrifuged at 1,500 x g for 10 minutes. The plasma was promptly aliquoted and stored at −70°C for subsequent analysis. An interview was conducted with questions regarding cerebral symptoms in the last three days, covering detailed questions regarding headache and visual disturbances, including scotomas, blurred vision, and diplopia.

Data regarding infant birth weight, gestational age at birth and mode of delivery was obtained from the women's medical records. Small for gestational age (SGA) was defined as a birth weight < -2 SDs from the sex-specific national reference curve.[158] Gestational age was estimated by an early second trimester ultrasound.

Paper III

Paper III was a national register-based cohort study. Women with singleton pregnancies who gave birth to their first infant at 22+0 gestational weeks or later, between January 1, 2005, and December 31, 2018, were indentified in the MBR (Figure 9). Women with chronic hypertension (checkbox in MBR and/or ICD-code: O10, I10-I13, and I15 in MBR) at the time of the index pregnancy were excluded to avoid potential confounding effects on the outcomes, as pre-existing cardiovascular dysfunction can influence pregnancy-related results. Women diagnosed with any of the neurological

outcome diagnoses (migraine, headache, epilepsy, sleep disorder, and mental fatigue), as recorded in the National Patient Register either before or during pregnancy, or within 42 days postpartum, were also excluded to avoid confounding symptoms related to the index pregnancy. All births were standardized to the 15th of the respective month to protect confidentiality. Follow-up started 42 days after the set birthdate.

Information on maternal sociodemographic and clinical characteristics was obtained from the MBR and included early pregnancy age, smoking, cohabitation status, pregestational diseases, and gestational diabetes. Early pregnancy maternal body mass index (BMI) was calculated by the equation $weight\ (kg)/height\ (m)^2$ using measured weight and generally self-reported height. BMI values < 15 or > 55 were replaced by missing values, since it was assumed that they were due to incorrectly registered values in the register. Data on the highest achieved education level until 2020 was retrieved from the Education Register, irrespective of the date and year of their first birth. Country of birth data was obtained from the Total Population Register.

Data on infant characteristics were sourced from the MBR and included gestational age at birth, sex, birth weight, SGA, and stillbirth. Gestational age was established by either a first or early second trimester ultrasound for > 90% of women in this cohort, secondly by an estimation from last menstrual period (LMP) data in the maternal health care (3,9%) or by estimation by the delivery ward (4,6%) if no previous ultrasound or LMP data was at hand. Preterm birth was defined as birth before 37+0 weeks of gestation. SGA was defined as described in Paper I and II. Stillbirth included both antenatal and intrapartum stillbirths.

Paper IV

Paper IV was a national register-based cohort study. Women who gave birth to their first infant (singleton ≥ 22+0 gestational weeks) in Sweden from January 1, 2016, to December 31, 2017, were eligible to the study and identified in the MBR (Figure 11). Women < 18 years of age at childbirth, and women who emigrated or died within two years postpartum were excluded.

Maternal demographic information such as age at delivery, early pregnancy BMI (the same formula was used to calculate BMI as in Paper III and BMI values < 15 or > 60 were replaced by missing values), smoking, and country of birth were collected from the MBR. Data on pregestational disorders was retrieved from predefined checkboxes in the MBR and/or diagnostic codes according to ICD-10 from the MBR and/or the National Patient Register. Data on the highest achieved education level until 2020 was gathered from the Education Register.

All outcome variables regarding sick leave were sourced from the LISA database, which also contributed with information on some of the exclusion criteria.

Exposures

Paper I and II

The exposure group consisted of pregnant women with preeclampsia (n=28). Preeclampsia was defined as SBP ≥ 140mmHg and/or DBP ≥ 90 mmHg measured on two subsequent occasions at least 6 hours apart and proteinuria ≥ 2+ on a dipstick or ≥ 300mg/24h in a urine collection. Within the preeclampsia group, 13 women had early-onset preeclampsia and 15 women had late-onset preeclampsia, as previously defined. Preeclampsia with severe features was defined as SBP ≥ 160 mmHg and/or DBP ≥ 110 mmHg, or if HELLP syndrome was present.[15]

Two control groups were recruited; women with normotensive pregnancies (n=28), matched for maternal age and gestational age at recruitment, and non-pregnant women (n=16). A normotensive pregnancy was defined as one in which the woman remained normotensive throughout its duration. The pregnancy also had to result in term delivery (≥ 37+0 gestational weeks) of an infant whose birth weight was within ± 2 SD of the mean for their gestational age and sex. The non-pregnant group consisted of both parous and nulliparous women. In addition to the general exclusion criteria, described earlier, a previous history of HDP was an additional exclusion criterion for the control group.

Paper III

Three exposure groups were established and identified by ICD-codes in the MBR; gestational hypertension (ICD-code: O13), preeclampsia without eclampsia (ICD-code: O14), and eclampsia (ICD-code: O15). Gestational hypertension was clinically defined as new-onset elevated SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg, measured on two subsequent occasions > 4 hours apart, occurring after 20 weeks of gestation and in the absence of proteinuria. During the study period preeclampsia was defined as gestational hypertension accompanied by proteinuria (≥ 300 mg/24 hours, ≥ 2+ on dipstick, or 1+ on dipstick on two separate occasions with > 4 hours apart) according to Swedish clinical practice.[159] Eclampsia was defined as tonic-clonic seizures without other etiology, accompanied by a diagnosis of preeclampsia.

In a sub analysis, all women with preeclampsia (with or without eclampsia) were merged and then assigned to one of two groups; preterm (delivery < 37+0 gestational weeks) or term (delivery ≥ 37+0 weeks) preeclampsia. Women with missing data on gestational age and women with gestational hypertension were excluded from the sub analysis.

For all analyses, the control group consisted of women with normotensive pregnancies.

Paper IV

The primary exposure group was all women with preeclampsia. Women were identified by ICD-coded diagnoses in the MBR and included; preeclampsia (ICD-code: O14), superimposed preeclampsia (ICD-code: O11), eclampsia (ICD-code: O15), and HELLP syndrome (ICD-code: O14.2). According to Swedish clinical practice during the study time, preeclampsia was defined as gestational hypertension accompanied by proteinuria (defined as in Paper III).[159]

Two sub analyses were conducted. In the first, women with preeclampsia were divided into three groups: women with preeclampsia without eclampsia or HELLP syndrome, women with eclampsia, and women with HELLP syndrome. Women who were diagnosed with both eclampsia and HELLP syndrome according to the MBR, were categorized in the eclampsia group. At the study time, eclampsia was defined as tonic-clonic seizures without other etiology in women diagnosed with preeclampsia, according to clinical practice in Sweden. HELLP syndrome was defined as hemolysis (haptoglobin < 0.25 g/L or LD < 600 U/L / > 10 μkat), elevated liver enzymes (AST or ALT ≥ 1.2 μkat/L), and low platelets (< 100 $x10^9$ /L) in pregnancy, with or without high blood pressure.

In the second sub analysis, women with preeclampsia were assigned to two groups: women with preterm preeclampsia (delivery < 37+0 gestational weeks) and women with term preeclampsia (delivery ≥ 37+0 weeks). Women with missing data on gestational age were excluded from the sub analysis.

For all analyses, the control group consisted of women who did not develop preeclampsia, however, including women with chronic and gestational hypertension.

Outcomes

For Paper I and II, frozen plasma was shipped to the University of Bío-Bío in Chillán, Chile, thawed and used for the *in vitro* BBB experiments previously described.

Paper I

Outcomes were change in TEER (ΔTEER) and permeability to FITC-Dextran 70kDa before and after exposure of cells to plasma from the study participants, for assessment of BBB integrity. The mRNA expression of VEGFR2 was measured with qPCR.

Analyses to further explore possible underlying mechanisms to BBB injury were also performed, and described in more detail in Paper I.

Paper II

TEER values from Paper I were used for the assessment of BBB integrity.

Thawed plasma from the same study participants as in the *in vitro* experiments was used for analysis of cerebral biomarkers. NfL and tau concentrations were measured with Simoa in Mölndal, Sweden, wheras NSE and S100B concentrations were measured with ELISA in Uppsala, Sweden.

Correlation between TEER and cerebral biomarkers was the main outcome.

Paper III

The primary outcome was a composite of the five neurological disorders migraine (ICD-code: G43), headache (ICD-code: G44), epilepsy (ICD-code: G40), sleep disorder (ICD-code: G47) and mental fatigue (ICD-code: F48 neurasthenia). In the case of multiple morbidities, the first occurring diagnosis was used.

As a secondary outcome the five diagnoses were analyzed as individual components. Outcomes were identified by their respective ICD-diagnoses in the National Patient Register.

Follow-up started 42 days after the set birthdate, and all women had to be available for follow-up for at least one year.

Paper IV

The primary outcome was the total number of days on sick leave exceeding 14 days, accumulated from all sick leave absences exceeding 14 days, in the second year postpartum.

Secondary outcomes were occurrence of any sick leave episodes exceeding 14 days in the second year postpartum, the total number of days on sick leave analyzed only among women who had at least one sick leave episode in the second year postpartum, and the number of sick leave episodes exceeding 14 days among women who had at least one sick leave episode in the second year postpartum.

All outcome variables were retrieved from the LISA database.

Statistical analysis

Paper I

Background characteristics were compared between groups by student's t-test or Chi-square test. For the BBB permeability analyses, differences between groups were assessed using the nonparametric Mann Whitney U-test. In case of statistical significance, the Bonferroni post hoc test was used.

Data and statistical analyses were performed using SPSS version 25, GraphPad Prism 6.00 (GraphPad Software, CA), and R version 3.6.1 using the add-on package mgcv.

Paper II

Background characteristics were compared by one-way ANOVA, Chi-Square or Kruskal–Wallis test, and the significance level was set at 0.05.

Comparisons of plasma concentrations of the cerebral biomarkers (NfL, tau, NSE and S100B) between groups were performed using non-parametric methods; the Kruskal-Wallis test for overall differences and the Mann-Whitney U-test for pairwise comparisons. In case of statistical significance, a Bonferroni post-hoc test was used.

Associations between concentrations of the cerebral biomarkers and TEER values were analyzed with a cumulative probability model. The model was adjusted for baseline TEER, and for the confounders maternal age, parity and BMI. The confounders were identified with a directed acyclic graph (DAG). The model was subsequently adjusted to a model where all associations were linear.

Data and statistical analyses were performed with IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp, Armonk, NY, USA), GraphPad Prism 6.00 (GraphPad Software, San Diego, CA, USA) and R version 3.6.1 with the add-on package rms.

Paper III

Associations between the exposure groups and the composite neurological outcome were explored through Cox regression analysis. A DAG was constructed to obtain systematic representation of relationships between HDP and the neurological disorders. Age at childbirth, early pregnancy BMI and smoking, pregestational and gestational diabetes, renal disease, SLE, maternal education level and country of birth were identified as possible confounders. Risks were expressed as crude and adjusted Hazard Ratios (HRs) with 95% confidence intervals (CI).

Due to confidentiality reasons, the MBR only provides monthly data for births, not exact dates, therefore, all births were set to the 15th of each month. Follow-up began 42 days postpartum, i.e., 27-57 days after the actual date of birth, and ended at the first occurrence of a neurological event, death, or emigration, or at the end of the study period on December 31, 2019. The minimum time of follow-up was one year postpartum and the longest possible follow-up time was 15 years. Data was analyzed using time-to-event methods for the different exposure groups, and incidence rates were calculated.

Associations between gestational hypertension, preeclampsia (without eclampsia), and eclampsia and the separate neurological diagnoses (migraine,

headache, epilepsy, sleep disorder, and mental fatigue) were explored with the same methods.

Associations between term and preterm preeclampsia, and the composite and separate neurological outcomes were explored through Cox regression analysis.

Statistical analyses were performed using SPSS software (version 28.0, IBM Corp., Armonk, NY).

Paper IV

Comparisons between exposure groups were conducted using both unadjusted and adjusted analyses to evaluate both the primary and secondary outcomes related to sick leave. Unadjusted analyses were performed using Welch's t-test for continuous variables, the Farrington-Manning test for binary variables, and Poisson regression for count variables. To illustrate potential confounding factors of preeclampsia and sick leave, a DAG was created. Adjustment was suggested for maternal age at delivery, early pregnancy BMI, smoking, country of birth, cohabitation status, education level, diabetes (both pregestational and gestational), chronic kidney disease, SLE, and for the number of sick leave days exceeding 14 days in the calendar year that occurred two years prior to childbirth. Adjusted analyses were performed using augmented inverse probability weighting, which combines outcome regression with inverse probability weighting for group membership to obtain robust inferences. Results from the unadjusted and adjusted analyses were presented as mean difference and proportion difference, as appropriate, along with 95% CIs.

Statistical analyses were performed using SAS/STAT® Software, version 9.4 (SAS Institute Inc. Cary, NC, USA) and using R version 4.4.1 (R Core Team, Vienna Austria).

Results

Paper I

The pregnant study groups in Paper I and II were of similar maternal age and gestational age at inclusion. Women with preeclampsia had higher BMI values and were more frequently nulliparous compared to women in the control groups. About one-third of women in the preeclampsia group had severe features (severe headache and/or visual disturbances) and 79% had antihypertensive treatment at inclusion, but none experienced acute neurological complications or cerebral edema on MRI. At delivery, 57% had developed severe preeclampsia, but no eclampsia cases occurred, and none received $MgSO_4$ prophylaxis.

A greater reduction in TEER (i.e., a larger ΔTEER) was seen in cell monolayers exposed to plasma from women with preeclampsia (8.2, IQR 5.8–10.2) compared with cells exposed to plasma from women with normotensive pregnancies (5.2, IQR 2.6–8.2) and non-pregnant women (4.0, IQR 1.4–5.3), (Figure 6A). Cell monolayers exposed to plasma from women with preeclampsia demonstrated increased permeability to 70 kDa FITC-Dextran (3.4, IQR 1.5–10.0), compared with cells exposed to plasma from women with normotensive pregnancies (1.2, IQR 0.6–1.9), (Figure 6B).

In a sub analysis, cell monolayers exposed to plasma from late-onset preeclampsia demonstrated a larger ΔTEER and a more substantial increase in permeability to FITC-Dextran compared with both early-onset preeclampsia and late normotensive pregnancy (Figure 1b and 1d in Paper I).

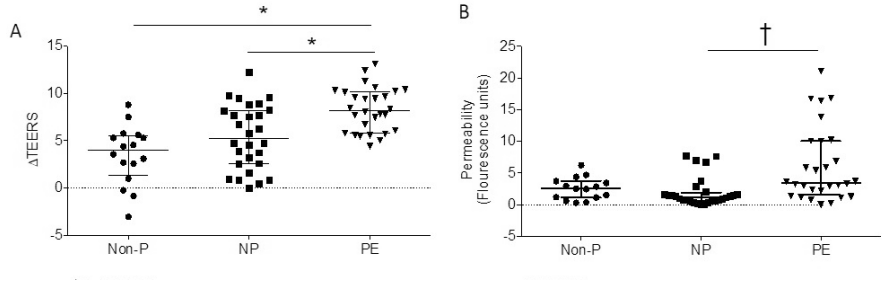

Figure 6A-B. Plasma exposed human brain endothelial cells. Preeclampsia (PE), normotensive pregnancy (NP), and non-pregnant (Non-P). Medians with interquartile range (IQR). *P < 0.0001; †P < 0.01.
(A) Reduction in transendothelial electrical resistance (ΔTEER)
(B) Increase in permeability to Dextran (fluorescence units).

Cell monolayers exposed to plasma from women with preeclampsia expressed higher mRNA levels of VEGFR2 (1.0, IQR 0.5–1.6), compared with cells exposed to plasma from normotensive pregnancies (0.5, IQR 0.1–1.0) (Figure 2 in Paper I).

Paper II

Plasma concentrations of the cerebral biomarker NfL were higher in women with preeclampsia (8.85, IQR 6.78–12.65 ng/L) compared with normotensive pregnancies (5.25, IQR 3.93–7.63 ng/L) and non-pregnant controls (5.65, IQR 4.83–6.40 ng/L), (Figure 7a). Plasma concentrations were also higher in preeclampsia than in normotensive pregnancy of tau (2.90, IQR 2.40–4.35 ng/L *vs* 2.40, IQR 1.80–2.58 ng/L, Figure 7b), NSE (3.50, IQR 2.84–4.55 µg/L *vs* 2.37, IQR 1.93–2.85 µg/L, Figure 7c), and S100B (0.08, IQR 0.05–0.10 µg/L *vs* 0.05, IQR 0.03–0.08 µg/L, Figure 7d).

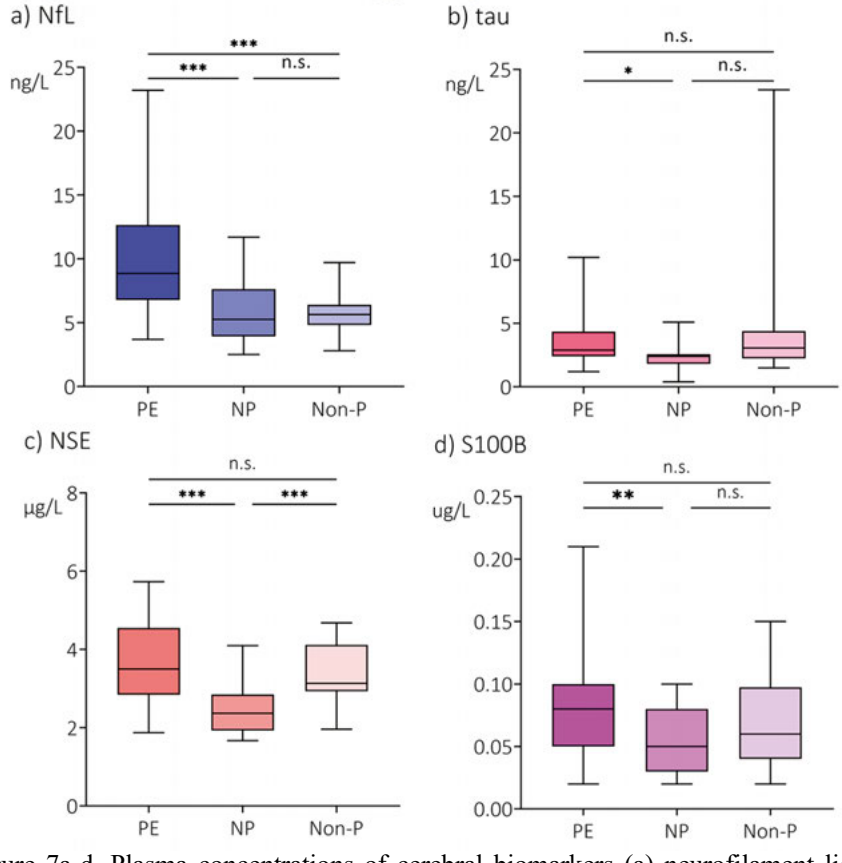

Figure 7a-d. Plasma concentrations of cerebral biomarkers (a) neurofilament light (NfL), (b) tau, (c) neuron specific enolase (NSE) and (d) S100B. Preeclampsia (PE), normotensive pregnancy (NP) and non-pregnant (Non-P). Medians with interquartile range (IQR). *p < 0.05; **p < 0.01; ***p < 0.001; n.s. = non-significant.

Associations between cerebral biomarkers (Figure 7a-d) and changes in TEER (Figure 6A) are presented in Figure 8. Higher plasma concentrations of NfL correlated with a greater reduction in TEER after monolayers of cells were exposed to plasma ($p < 0.01$). No associations were found between tau, NSE, or S100B and TEER.

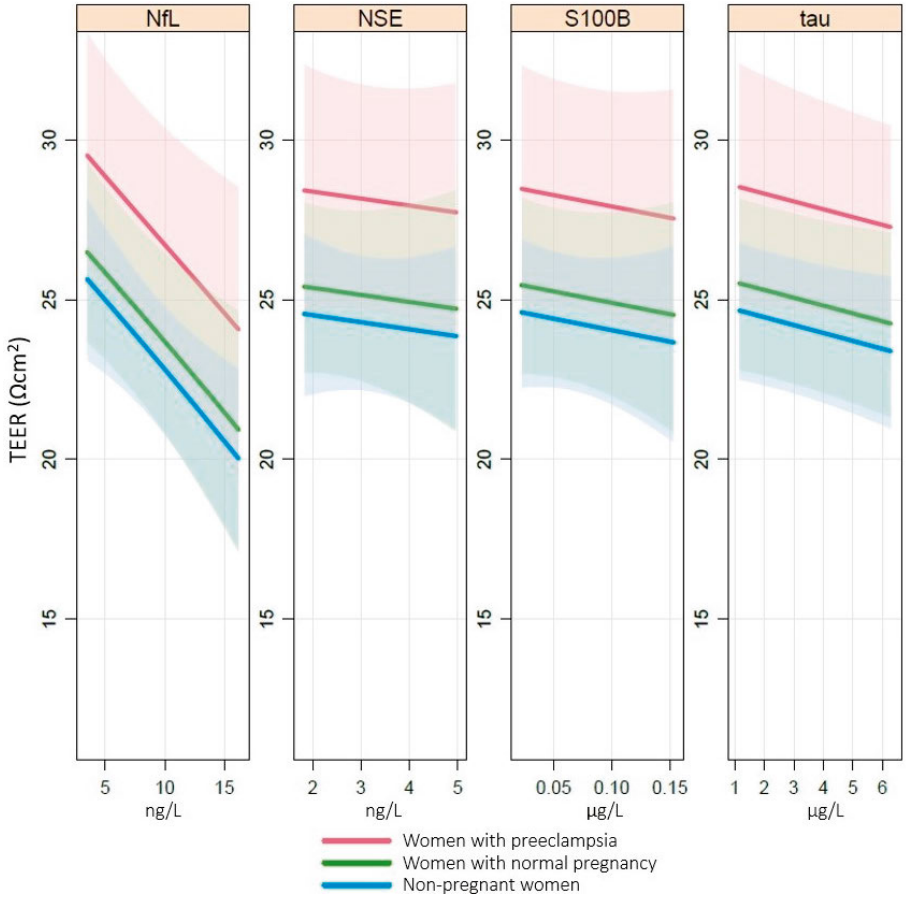

Figure 8. Associations between transendothelial electrical resistance (TEER (Ωcm2)) and cerebral biomarkers neurofilament light (NfL), tau, neuron-specific enolase (NSE) and S100B in plasma analyzed with a cumulative probability model stratified by group and adjusted for baseline TEER, body mass index, parity and maternal age. NfL $p < 0.01$, tau p = n.s., NSE p = n.s., S100B p = n.s.

Paper III

The final study cohort included 648,385 women (Figure 9). Among them, 94.1% of women had a normotensive pregnancy, 1.7% had gestational hypertension, 4.1% had preeclampsia without eclampsia, and 0.1% had eclampsia.

Among women with preeclampsia, 21.1% delivered preterm and 78.9% delivered at term.

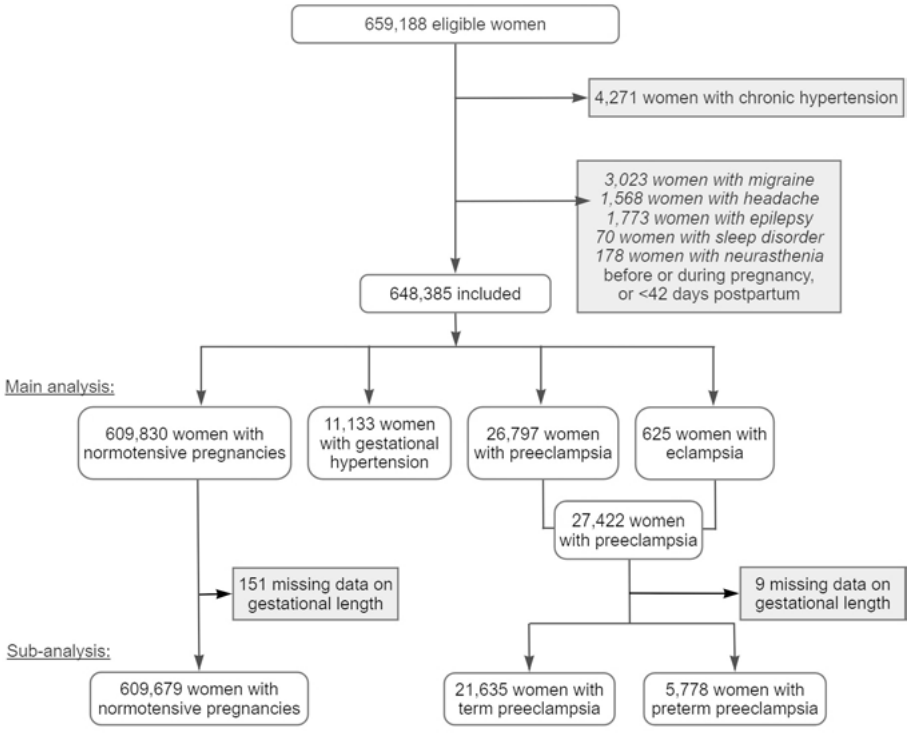

Figure 9. Flowchart of study participants in Paper III.

Women with gestational hypertension and preeclampsia had higher mean BMI and were more likely to have pregestational disorders, such as diabetes, kidney disease, and SLE, than women with normotensive pregnancies.

The total follow-up time of the study population was over 5 million person-years, with a mean follow-up time of 7.7 years per individual.

Of the main exposure groups, women with eclampsia had the highest cumulative event rate for the composite neurological outcome (Figure 10). Within four years postpartum, approximately 2% of them experienced a neurological event. The corresponding incidence for women with preeclampsia and gestational hypertension was 1%. Subsequently, the incidence in all exposure groups increased by around 2% for every four years. Women with normotensive pregnancies had the lowest cumulative event rate.

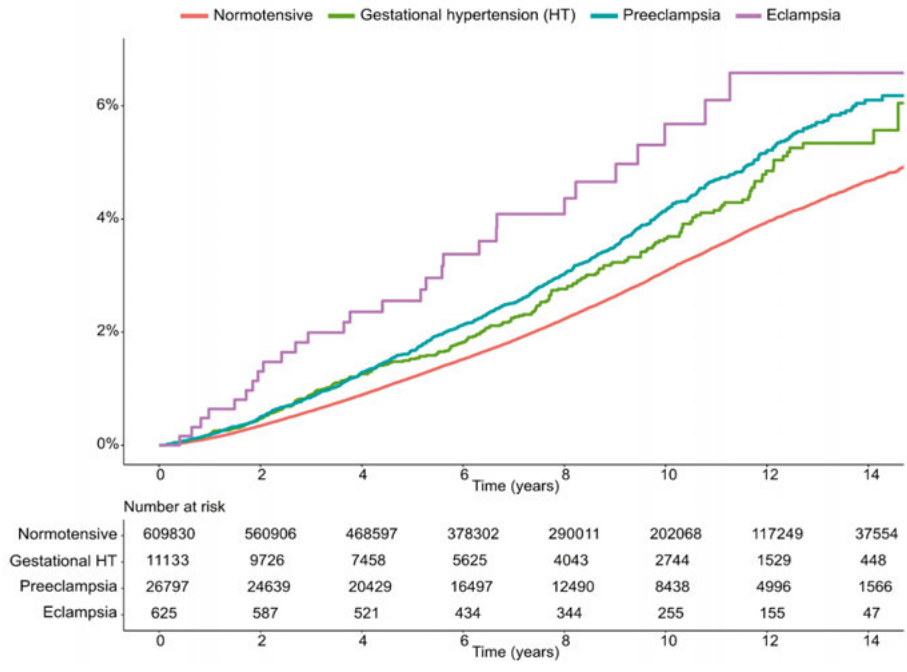

Figure 10. Kaplan-Meier curve illustrating the cumulative event rate for a composite of neurological disorders.

Higher incidence rates of developing a new-onset neurological disorder were found for women with gestational hypertension (3.6 per 1,000 person-years), preeclampsia (4.0 per 1,000 person-years), and eclampsia (5.4 per 1,000 person-years), compared with women with normotensive pregnancies (3.0 per 1,000 person-years), (Table 2).

Compared with women with normotensive pregnancies, there was an association with increased risk of the composite outcome for women with gestational hypertension (aHR 1.27, 95% CI 1.12–1.45), women with preeclampsia (aHR 1.32, 95%CI 1.22–1.42), and women with eclampsia (aHR 1.70, 95% CI 1.16–2.50), (Table 2). After imputation of missing confounding data, the aHR remained essentially unchanged (Table 2).

Compared with women with normotensive pregnancies, women with gestational hypertension (aHR 1.39, 95% CI 1.19–1.63) and preeclampsia (aHR 1.25, 95% CI 1.13–1.38) were associated with an increased risk of migraine (Table 2). Preeclampsia was also associated with an increased risk of headache (aHR 1.51, 95% CI 1.33–1.71) and epilepsy (aHR 1.32, 95% CI 1.07–1.63). The strongest association was observed in women with eclampsia, who had a fivefold increased risk (aHR 5.31, 95% CI 2.85–9.89) for epilepsy (Table 2).

In the sub analysis, there was an association with increased risk for the composite neurological outcome for women with preterm (aHR 1.54, 95% CI 1.34–1.79) and term (aHR 1.27, 95% CI 1.17–1.38) preeclampsia compared

with women with normotensive pregnancies. Both preterm and term preeclampsia were associated with increased risks of migraine and headache, with higher aHRs for preterm than term preeclampsia. Women with term preeclampsia were also associated with increased risk of epilepsy (aHR 1.41, 95% CI 1.13–1.77), (Table 3 in Paper III).

Table 2. Risk of developing a neurological disorder after a first pregnancy complicated by gestational hypertension, preeclampsia, and eclampsia, main analysis (n=648 385).

	Normotensive pregnancy (*n*=609 830)	**Gestational Hypertension (*n*=11 133)**	**Preeclampsia[a] (*n*=26 797)**	**Eclampsia (*n*=625)**
Primary outcome				
Composite of neurological disorders[b]				
Total person-years of follow-up	4 728 612	74 614	205 470	5330
No. of events (%)	14 221 (2.3)	269 (2.4)	828 (3.1)	29 (4.6)
Event rate per 1,000 person-years	3.0	3.6	4.0	5.4
HR (95% CI), crude	1.0	1.24 (1.10-1.40)	1.35 (1.25-1.44)	1.77 (1.23-2.55)
aHR (95% CI), complete dataset	1.0	1.27 (1.12-1.45)	1.32 (1.22-1.42)	1.70 (1.16-2.50)
Secondary outcomes				
Migraine				
No. of events (%)	8309 (1.4)	179 (1.6)	458 (1.7)	13 (2.1)
Event rate per 1,000 person-years	1.7	2.4	2.2	2.4
HR (95% CI), crude	1.0	1.41 (1.22-1.64)	1.27 (1.16-1.40)	1.34 (0.78-2.31)
aHR (95% CI), complete dataset	1.0	1.39 (1.19-1.63)	1.25 (1.13-1.38)	1.47 (0.86-2.54)
Headache				
No. of events (%)	4473 (0.7)	76 (0.7)	283 (1.1)	6 (1.0)
Event rate per 1,000 person-years	0.9	1.0	1.4	1.1
HR (95% CI), crude	1.0	1.12 (0.89-1.40)	1.46 (1.29-1.65)	1.15 (0.52-2.55)
aHR (95% CI), complete dataset	1.0	1.27 (1.01-1.61)	1.51 (1.33-1.71)	1.00 (0.42-2.40)
Epilepsy				
No. of events (%)	1671 (0.3)	29 (0.3)	109 (0.4)	12 (1.9)
Event rate per 1,000 person-years	0.4	0.4	0.5	2.2
HR (95% CI), crude	1.0	1.13 (0.78-1.62)	1.50 (1.24-1.82)	6.26 (3.55-11.05)
aHR (95% CI), complete dataset	1.0	1.14 (0.78-1.66)	1.32 (1.07-1.63)	5.31 (2.85-9.89)
Sleep disorder				
No. of events (%)	352 (0.1)	3 (0.0)	23 (0.1)	1 (0.2)
Event rate per 1,000 person-years	0.1	0.0	0.1	0.2
HR (95% CI), crude	1.0	0.57 (0.18-1.78)	1.51 (0.99-2.30)	2.39 (0.34-17.04)
aHR (95% CI), complete dataset	1.0	0.59 (0.19-1.84)	1.28 (0.81-2.02)	2.43 (0.34-17.29)

aHR; adjusted Hazard ratios; CI, Confidence intervals; HR, Hazard ratios
aHR = Adjusted for pregestational and gestational diabetes, systemic lupus erythematosus and renal disease, early pregnancy body mass index, age at childbirth, smoking habits, maternal education level and country of birth.
[a] Preeclampsia without eclampsia
[b] The composite outcome includes migraine, headache, epilepsy, sleep disorder, and mental fatigue

Paper IV

The final study cohort included 95,716 women (Figure 11). Preeclampsia was diagnosed in 4.5% of women. Among them, 1.4% experienced eclampsia and 4.1% experienced HELLP syndrome. Among women with preeclampsia, 21.1% delivered preterm and 78.9% delivered at term.

Women who experienced preeclampsia had a higher BMI and a higher prevalence of chronic conditions at inclusion. They also had more sick leave days two calendar years prior to the index childbirth compared to the reference group (10.0 days, SD 47.0 *vs* 6.7 days, SD 37.05).

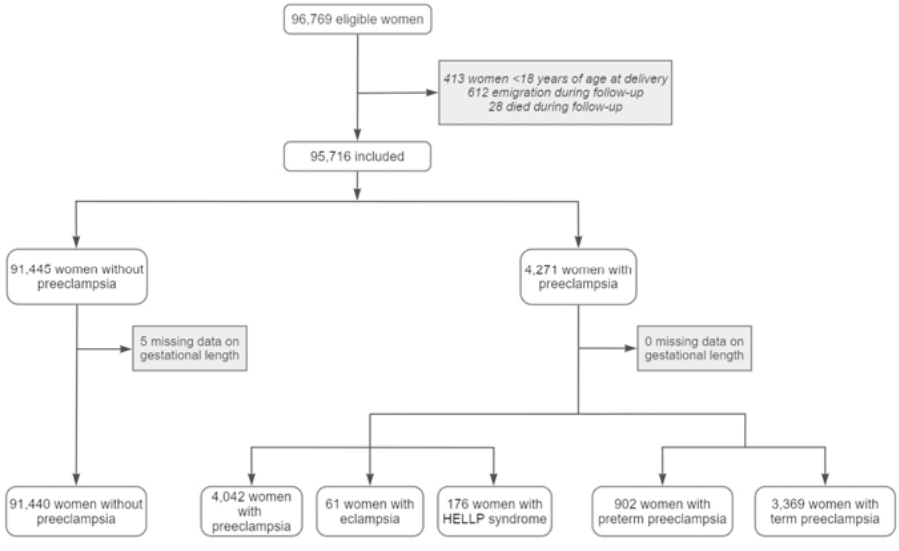

Figure 11. Flowchart of study participants in Paper IV.

Women who experienced preeclampsia had more sick leave days in the second year postpartum compared with the reference group (15.6 days, SD 49.9, *vs* 11.0 days, SD 40.9; mean difference (MD) 4.6 days, 95% CI 3.1–6.1), (Table 3). After adjusting for confounders, the results remained unchanged (adjusted mean difference (aMD) 4.6 days, 95% CI 3.1–6.1). The absolute number of sick leave days was highest among women with eclampsia, but did not reach statistical significance compared with the reference group (25.1 days, SD 76.1 *vs* 11.0 days, SD 40.9; aMD 13.9 days, 95% CI -5.0–32.9). When categorized by gestational age, women with preterm preeclampsia had the highest number of sick leave days compared with the reference group (19.8 days, SD 60.7 *vs* 11.0 days, SD 40.9; aMD 8.7 days, 95% CI 4.8–12.7), (Table 3).

Women with preeclampsia were more often on sick leave in the second year postpartum, 21.7% compared with 16.5% of women in the reference group (absolute percentage difference 5.3%, 95% CI 4.0–6.5). The frequency of women taking sick leave was numerically higher (26.2%) among women

with eclampsia, but did not reach statistical significance compared with the reference group. Women with preterm preeclampsia had the highest frequency of sick leave episodes compared with the reference group, when categorized by gestational age (Table 4).

Table 3. Mean (SD) number of days on sick leave exceeding 14 days in the second year postpartum.

			Mean difference (95% CI) *vs* reference			
Study group	N	Mean (SD)	Unadjusted[a]	p-value	Adjusted[b]	p-value
No preeclampsia (reference)	91,445	11.0 (40.9)	ref.		ref.	
Preeclampsia	4,271	15.6 (49.9)	4.6 (3.1–6.1)	<.001	4.6 (3.1–6.1)	<.001
Preeclampsia without eclampsia or HELLP	4,042	15.6 (49.5)	4.6 (3.0–6.1)	<.001	4.6 (3.1–6.1)	<.001
Eclampsia	61	25.1 (76.1)	13.9 (-5.6–33.4)	0.16	13.9 (-5.0–32.9)	0.15
HELLP syndrome	176	16.0 (58.0)	4.8 (-3.8–13.5)	0.27	4.8 (-3.7–13.4)	0.27
Preterm* preeclampsia	902	19.8 (60.7)	8.7 (4.7–12.7)	<.001	8.7 (4.8–12.7)	<.001
Term preeclampsia	3,369	14.5 (46.6)	3.4 (1.8–5.0)	<.001	3.4 (1.8–5.0)	<.001

CI, confidence interval; HELLP, hemolysis, elevated liver enzymes, and low platelet counts; SD, standard deviation

*Birth before 37 gestational weeks.

[a] Unadjusted analyses used the Welch's t-test to account for unequal variances between groups.

[b] Adjusted analyses were performed using augmented inverse probability weighting, adjusting for the number of sick leave days two years prior to childbirth, maternal age at delivery, body mass index, country of birth, cohabitation status, smoking during early pregnancy, education level, diabetes (both pre-gestational and gestational), chronic kidney disease, and systemic lupus erythematosus.

Table 4. Occurrence of any sick leave episode exceeding 14 days in the second year postpartum.

		Percentage difference (95% CI) *vs* reference			
Study group	N (%)	Unadjusted[a]	p-value	Adjusted[b]	p-value
No preeclampsia (reference)	15,062 (16.47)	ref.		ref.	
Preeclampsia	928 (21.73)	5.3 (4.0–6.5)	<.001	5.3 (4.0–6.5)	<.001
Preeclampsia without eclampsia or HELLP	875 (21.65)	5.2 (3.9–6.5)	<.001	5.2 (3.9–6.4)	<.001
Eclampsia	16 (26.23)	9.5 (-1.5–20.6)	0.046	9.5 (-1.5–20.6)	0.091
HELLP syndrome	41 (23.30)	6.6 (0.4–12.9)	0.019	6.6 (0.4–12.8)	0.038
Preterm preeclampsia	215 (23.84)	7.2 (4.4–10.0)	<.001	7.2 (4.4–10.0)	<.001
Term preeclampsia	713 (21.16)	4.6 (3.2–6.0)	<.001	4.6 (3.2–6.0)	<.001

CI, confidence interval; HELLP, hemolysis, elevated liver enzymes, and low platelet counts

Number and percentage of women with any sick leave episode in the second year postpartum, along with absolute risk difference (difference in proportions) compared to the reference group and 95% CIs.

*Birth before 37 gestational weeks.

[a] Unadjusted analyses used the Farrington-Manning test for the difference in proportions.

[b] Adjusted analyses were performed using augmented inverse probability weighting, accounting for any sick leave episode two years before childbirth, maternal age at delivery, body mass index, country of birth, cohabitation status, smoking during early pregnancy, education level, diabetes (both pre-gestational and gestational), chronic kidney disease, and systemic lupus erythematosus.

Discussion

The aim of this translational thesis was to study the pathophysiology behind cerebral complications of preeclampsia, focusing on the blood-brain barrier, and to investigate the impact of preeclampsia and eclampsia on women's neurological health and sick leave in the years following childbirth.

Main findings

The first study demonstrated that plasma from women with preeclampsia altered the BBB integrity in a human *in vitro* model by a greater reduction in TEER and increased permeability to 70 kDa FITC-Dextran, compared with plasma from women with normotensive pregnancies and non-pregnant women.

The second study demonstrated a correlation between higher concentrations of the cerebral biomarker NfL in plasma and a larger decrease in TEER in the human *in vitro* BBB. Tau, NSE, and S100B were not associated with TEER.

The third study established that women with gestational hypertension, preeclampsia, and eclampsia had an association with increased risk of developing a composite of neurological disorders in the years following their first childbirth, compared with women who had normotensive pregnancies. The strongest association was found between eclampsia and epilepsy, with a fivefold increase in risk.

The fourth study found that women who experienced preeclampsia in their first pregnancy had more long-term sick leave in their second year postpartum compared to women without preeclampsia.

Methodological considerations

Paper I and II

Study population

The study groups of Paper I and II were well-characterized, and plasma samples were taken for measurements of both cerebral biomarkers and *in vitro* BBB analyzes in the same women. However, most of the women in the

exposure group did not have severe preeclampsia, only one third of them reported neurological symptoms, i.e., headache or visual disturbances, and none developed severe CNS complications.[157] This is also consistent with the fact that concurrently performed MRI scans of the same women, as part of previously published studies, exhibited no signs of cerebral edema or ischemia.[157,160]

In vitro studies

In Paper I and Paper II, the use of a human *in vitro* model for investigating BBB alterations in preeclampsia was introduced for the first time, as opposed to previously used animal-based models.[9]

In vitro studies are considered a low-grade of evidence in research (Figure 12). However, translational studies can be hypothesis generating and may encourage future clinical research.[161] Even though *in vitro* models have their limitations and may not perfectly replicate *in vivo* conditions,[69] they are valuable in research on pregnant women, as some questions are hard to investigate *in vivo* due to ethical and practical constraints.

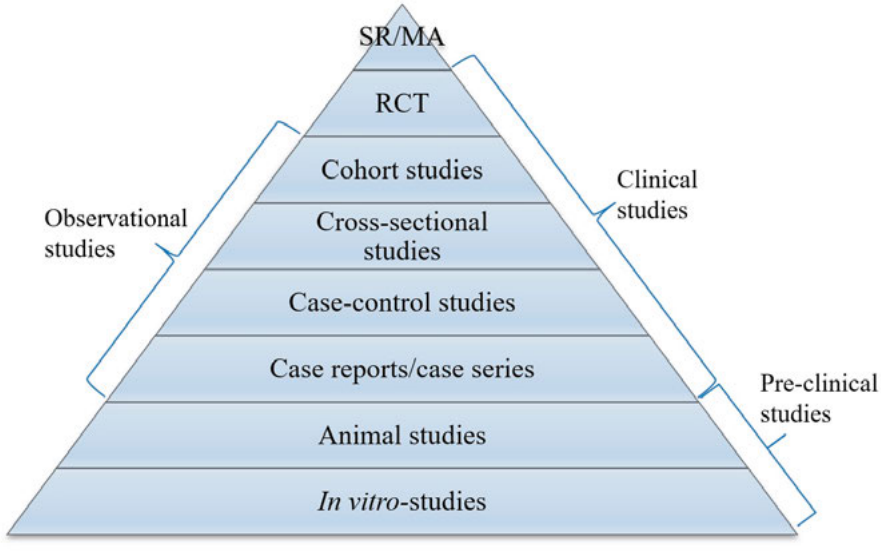

Figure 12. Evidence pyramid ranking study designs by strength. MA; meta-analysis, RCT; randomized controlled trial, SR; systematic review

To enhance reliability, researchers who performed the *in vitro* experiments for this thesis were blinded to the exposure groups, and all experiments were performed in duplicates.

Primary cell cultures might display a superior phenotype compared with an endothelial cell line, but the use of primary cell cultures from human brain tissue has several ethical concerns and limitations, thus an immortalized cell line provides a feasible alternative.

The hCMEC/D3 cell line is well characterized for brain endothelial phenotype, i.e., it mimics the human BBB well *in vitro*.[66] However, it does not originate from a woman with preeclampsia. Nevertheless, exposure of these cells to plasma from women with preeclampsia led to impairment of the BBB. This implies that potentially harmful endogenous molecules present in plasma in preeclampsia exerts a direct effect on the BBB. The hCMEC/D3 cell line could be used for further exploration of the pathophysiological mechanisms by which preeclampsia affects the BBB.

For these studies, a static *in vitro* model based on a 2D monoculture in transwells was used.[66] To improve the BBB phenotype, enhance tightness and increase TEER, adaptations could be made in several ways to better reflect *in vivo* conditions:

- Co-culturing with supportive cells, such as pericytes and/or astrocytes seeded either on the opposite side of the filter for direct interaction, or in the bottom of the lower compartment for indirect activation.[70,143]
- 2D-3D hybrid models where monolayers are seeded within a cylindrical channel in a gel.
- Dynamic 3D models with culturing of cells in the lumen of a cylinder, whereas supportive cells are co-cultured on the outside, meanwhile a pulsatile flow is generated in the cylinder to mimic capillaries in vivo.
- Microfluidic systems to more closely mimic the *in vivo* brain anatomy.[68,162]

Cerebral biomarkers

It is difficult to know whether the increased concentrations of NfL in women with preeclampsia reflect neuroaxonal injury and/or leakage across the BBB.[62] To address what these findings stand for, it would be valuable to perform more comprehensive analyses within the same population; BBB permeability assessments in an *in vitro* model, as well as cerebral biomarkers measurements in both plasma and CSF.

Paper III and IV

Study design

Both Paper III and IV are large register-based studies, including nearly 650,000 and 100,000 women, respectively. Due to the use of registry data, there was minimal loss to follow-up. The registries used in these studies are of high quality and have population-based coverage. Our exposures are considered to have good to very good validity in the Swedish MBR.[150] Validation studies of preeclampsia diagnoses in the Danish and Norwegian MBRs, which are similar to the Swedish MBR, have demonstrated high positive predictive values of 79% and 84%, respectively, and high specificity. However, sensitivity was lower, suggesting that mild cases may be underdiagnosed.[163,164]

Previous validations of the epilepsy diagnosis in the Swedish National Patient Register have demonstrated high accuracy. A recent register-based study reviewing medical records of patients hospitalized following traumatic brain injury found a 96.5% accuracy rate for the diagnosis, with 92% of the diagnosed individuals being prescribed antiseizure medication.[165] No validation studies specifically assessing the accuracy of migraine diagnoses in the National Patient Register were found.

Missing data was handled in a similar manner for both studies, using multiple imputation by chained equations. Five imputed datasets were generated due to large populations. Results were pooled using Rubin's rule. In Paper IV, BMI values from subsequent pregnancies were used to impute missing BMI values, if available. BMI was the variable with the largest amount of missing data, followed by data on cigarette smoking in early pregnancy and information on family situation. BMI is calculated from data on maternal length and measured weight in MBR, and some women may refrain from weighing themselves. Data on cigarette smoking is self-reported, and hence underreporting could be inflicting social desirability bias or misclassification bias. Likewise, the information about cohabitation is self-reported.

The lack of diagnoses from primary health care resulted in low absolute frequencies of some outcomes in Paper III. The epilepsy diagnosis is expected to be well covered in our dataset, given that all patients with a new onset of epilepsy/seizures should be referred to a neurologist or internal medicine specialist for clinical and neuroradiological/physiological examination before diagnosis.[166] The diagnoses migraine, headache, and sleep disorder are more prevalent in primary health care, which entails a risk of type 2 error due to the falsely low incidence in our dataset. We could only identify the most severe cases, i.e., women referred to specialized care.

To use composite outcomes in epidemiological studies can be considered debatable. Advantages may be that it could provide a more comprehensive measure of the total impact of disease burden and the power of a study can increase if outcomes are rare. However, it could also lead to an unequal weighting of the different components included, which may cause difficulties to interpret the results. In Paper III this was taken into account by also analyzing the separate neurological disorders as secondary outcomes. An association with increased risk for the composite outcome was found in all exposure groups, but when we separated the diagnoses gestational hypertension and preeclampsia leaned more towards migraine and headache, whilst eclampsia was more strongly associated with epilepsy, indicating that the underlying pathophysiological mechanisms may differ.

Utilizing data from the LISA database in Paper IV, we were constrained to analyzing sick leave on a calendar-year basis, a limitation that may not precisely reflect individual sick leave patterns. While we could determine the number of sick leave episodes per year, we were unable to link each episode to its specific duration. Consequently, it remains unclear whether a small

number of individuals account for a disproportionate share of total sick leave days due to extended absences. Additionally, our data extraction method could not identify individuals on parental leave during the outcome measurement period. This limitation may have led to inaccuracies in recording sick leave.

Study population

In both Paper III and IV, the main exposure was preeclampsia. The prevalence of preeclampsia in these study populations were 4.2% and 4.5%, respectively. This aligns with the prevalence reported in an earlier registry study of Swedish pregnant women from a different time period,[167] indicating representative study groups.

Register-based studies

Paper III and Paper IV of this thesis were population-based cohort studies. Cohort studies follow groups of individuals over time to observe associations between exposures and outcomes. Cohort studies are in the upper part of the evidence pyramid, in the top of the observational studies, however, still below randomized controlled trials, systematic reviews, and meta-analyses (Figure 12).[161] Direct causation cannot be established from cohort studies due to the potential influence of biases and confounding factors. To address this, we used DAGs to obtain a systematic overview of possible confounders associated with both the exposures and outcomes of Paper III and IV. In this way we could identify which confounders to adjust for in the analyses.

It can be challenging to study rare outcomes within a cohort study, even with large datasets. In Paper III mental fatigue (neurasthenia) had to be omitted from the separate analyses due to too few cases to generate meaningful results as a single outcome. However, it was still included in the composite as part of the overall neurological outcome.

The same challenge applies for the study of rare exposures. In Paper IV, the results of the sub analyses of women with eclampsia and HELLP syndrome did not reach statistical significance even though they had higher absolute numbers of sick leave days and episodes, than the women without preeclampsia. Our study cohort included only 61 cases of eclampsia, a condition that is rare in Sweden. To achieve statistical significance in future cohort studies, the number of study participants could be increased by extending the recruitment period to include more women or by prolonging the follow-up time to capture additional outcomes.

Research in context and research implications

The pathophysiology of cerebral complications in preeclampsia is multifactorial and still not completely understood.[10] Some of the acute cerebral complications seen in the disorder may arise through common pathways, whereas

others differ in mechanisms (Figure 13). The evidence of long-lasting cerebral changes seen in neuroradiological follow-up of these women indicate non-reversible damages to the cerebral parenchyma and perhaps also to the BBB,[58] which is strengthened by the many reports on long-term cerebrovascular risks.[6,7]

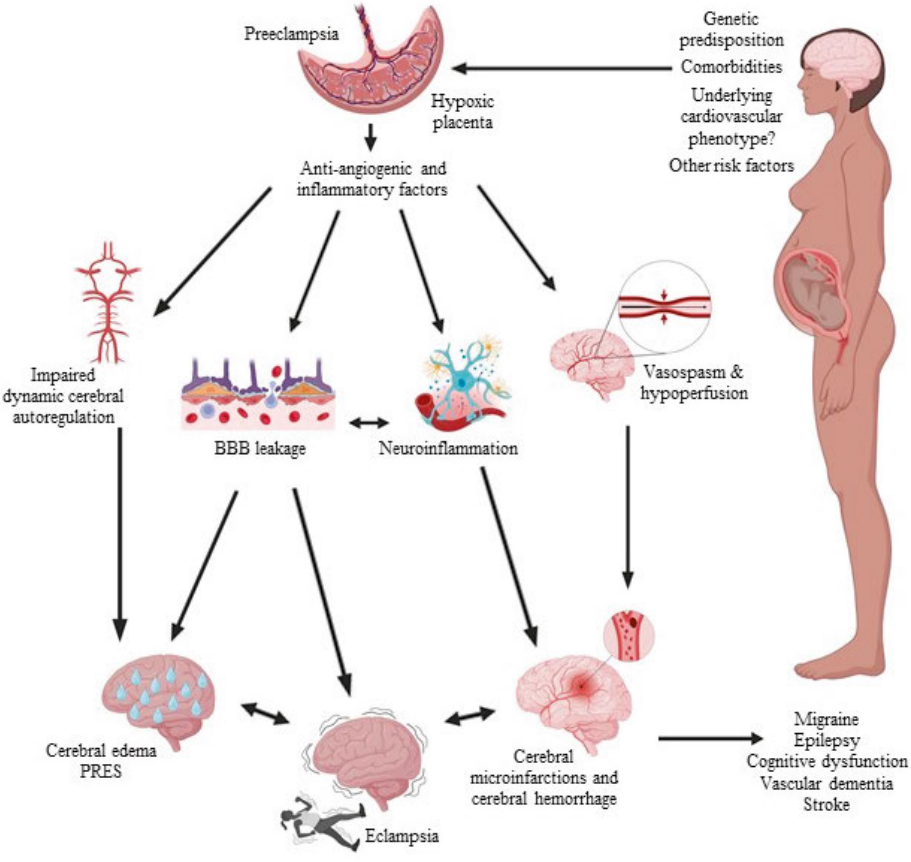

Figure 13. Suggested pathophysiological mechanisms of acute cerebral complications in preeclampsia. Created in BioRender.

The cerebral circulation and endothelial dysfunction have long been the focus of studies on women with preeclampsia. However, models for studying the brain in preeclampsia have traditionally included animal-based *in vitro* models, various preeclampsia-mimicking *in vivo* animal models, and human studies of hemodynamic conditions.[9,33,34,69,143,168]

Findings of cerebral endothelial dysfunction,[26,169] changes in BBB permeability (Paper I and II),[34] edema formation,[35,50] and increased biomarker concentrations in plasma (Paper II),[83] suggest structural changes that may inflict permanent damage. How and where in the brain these damages occur can likely affect which neurological symptoms the patient may subsequently

develop. Future studies should focus on the presumably different mechanisms of the separate neurological diagnoses.

Paper I

Measurements of TEER and permeability are indicators strongly associated with brain endothelium tightness. By the use of a human *in vitro* model of preeclampsia, findings of BBB impairment in animal-based models of preeclampsia[34,170] could be confirmed in Paper I, by demonstration of reduced TEER and increased permeability to 70kDa FITC-Dextran to indicate a damaged BBB. However, the findings of a greater reduction in TEER in late-onset preeclampsia contrast with those from an *in vitro* model based on rat cerebral veins, in which plasma from early-onset preeclampsia affected the BBB permeability to a greater extent.[48] This suggests that the cerebral response may differ between rodents and humans.

At the time of writing this thesis, a few additional publications have explored the hCMEC/D3 model in the context of preeclampsia. Both of them demonstrated that small extracellular vesicles extracted from plasma from women with preeclampsia, as well as from hypoxic placentas may be involved in the disruption of the BBB.[49,104] Other than that, no further studies based on entirely human *in vitro* BBB models were found for comparisons in pregnant populations with preeclampsia.

BBB disruption is reported in many pathological conditions, and at different levels of breakdown; from mild permeability changes and opening of tight junctions to changes in transport mechanisms and basement membrane degradation. It is suggested that more subtle BBB impairments might not be detected by using relatively large dextrans for permeability experiments, in those instances a smaller molecular weight marker such as sucrose would be better suited.[171]

The increased BBB permeability may be caused by the activation of VEGFR2,[172] which was demonstrated by a significant increase in the mRNA expression of VEGFR2. A study of *ex vivo* rat cerebral veins showed that plasma from women with preeclampsia increased the permeability, and that this effect was abrogated after inhibition of VEGFR2.[34] This supports the idea of VEGFR2 involvement in BBB disruption in preeclampsia.

To address the current shortage of human *in vitro* studies on preeclampsia, indirect signs of BBB disruption *in vivo* can be evaluated using various non-invasive MRI techniques[28,51] and more invasive CSF/plasma ratio measurements.[63] However, to gain a deeper understanding of the mechanisms underlying BBB impairment in preeclampsia, further basic science research is needed. This research should encompass both animal studies and the continued development of the recently introduced hCMEC/D3 preeclampsia model.

Paper II

The findings of increased plasma concentrations of cerebral biomarkers NfL, tau, NSE and S100B in women with preeclampsia, compared with women with normotensive pregnancies, are in line with previous publications from pregnant populations.[77-79,81] These four biomarkers have been extensively reported as useful circulating indicators of neurological conditions other than preeclampsia, such as neurodegenerative diseases, traumatic brain injury, and epilepsy.[62,74-76] Somewhat conflicting results regarding whether they reflect a BBB injury or not have been presented.[173,174]

In addition to increased plasma concentrations of NfL in preeclampsia compared with normotensive pregnancy, NfL was also higher in preeclampsia compared with non-pregnant women. Plasma concentrations of NfL, tau, and S100B did not differ when comparing normotensive pregnancy with non-pregnant women. Hence, pregnancy alone does not seem to have a major impact on circulating levels of these cerebral biomarkers. For a biomarker to be clinically reliable in predicting preeclampsia and its adverse outcomes, it is crucial that its concentrations are not elevated solely due to pregnancy itself. This ensures that the biomarker accurately reflects the presence of preeclampsia rather than normal physiological changes associated with pregnancy.

The finding of an association between higher circulating NfL concentrations in plasma and decreased TEER suggest that, at least in a pregnant population, BBB impairment could be reflected by NfL in plasma. This could be due to loss of ionic tightness (due to decreased claudin-5 expression in the cell membrane),[49] and possibly also by neuroaxonal injury with increased release of NfL.[175,176]

In a sub analysis of women with preeclampsia who expressed severe headache had higher concentrations of plasma NfL and a greater reduction in TEER, compared with women without neurological symptoms. This indicates that severity of disease might be associated with a more compromised BBB. The correlation between circulating concentrations of NfL and other cerebral biomarkers with disease severity and neurological symptoms (eclampsia, cortical blindness and stroke) is also supported by several previous studies.[79,80,83,177] With neuroaxonal damage, due to inflammatory, neurodegenerative, traumatic or vascular injury, NfL is released from the axons and can reach both the interstitial fluid of the CNS, and the blood.[74] NfL has been frequently used as a biomarker for neurodegenerative diseases,[75,178] and increased concentrations of NfL have been detected in both CSF and peripheral blood in patients with traumatic brain injury, compared with controls.[179] As such, NfL provides an accessible blood biomarker for neurological diseases.[180]

In preeclampsia, studies have reported increased plasma concentrations of NfL before the onset of disease.[77,78] Increased concentrations of NfL in both plasma and CSF in women with preeclampsia, compared with normotensive pregnant women, have also been demonstrated, with a strong correlation

between concentrations of NfL in plasma and CSF,[175] indirectly indicating BBB injury.[63] Increased plasma concentrations of NfL also correlated to worse cognitive function after delivery in women who experienced eclampsia, and in women with severe preeclampsia with pulmonary edema.[176] However, no other studies have explored a more direct association between circulating concentrations of cerebral biomarkers and BBB alterations in women with preeclampsia, as was done in Paper II.

Plasma concentrations of NSE, S100B and tau were not associated with TEER. Thus, other explanations than BBB damage should be considered as explanation for the higher concentrations of these biomarkers that were detected in the women with preeclampsia. These could include other transport pathways across the BBB, extracerebral existence, or hemolysis in the plasma samples.

Studies have shown that tau concentrations in plasma are increased in women before onset of disease in preeclampsia compared with normotensive controls,[77] and a further increase was seen in preeclampsia with severe features.[83]

Plasma concentrations of NSE are elevated during pregnancy before onset of preeclampsia, and they remain elevated one year postpartum.[81,82]

S100B is currently the most studied of these biomarkers when it comes to preeclampsia. Higher plasma concentrations have been reported in women with severe preeclampsia and eclampsia, and also one year postpartum.[79,82] Neurological symptoms, such as visual disturbances, have been correlated with increased S100B.[80,177]

Paper III

The association found between preeclampsia, and particularly eclampsia, and epilepsy is in line with a Canadian register-based study, in which a similar risk for seizure disorder was reported after eclampsia (aHR, 5.42, 95% CI 2.42-12.12).[118] Their follow-up time was similar to the one in Paper III. Interestingly, the association between preeclampsia and epilepsy differed between their study and the one conducted in Paper III (aHR, 1.96, 95% CI 1.21-3.17 compared with 1.32, 95% CI 1.07-1.63). This difference may be attributed to their use of a more comprehensive definition of seizure disorder and adjustment for other covariates. Beyond this study, only two case-series have reported on future risks of short- to medium-term neurological disorders after HDP. One study, that involved women with severe temporal lobe epilepsy who underwent surgery, suggested that eclampsia might be a risk factor for this type of epilepsy.[181] However, this was a small, uncontrolled, retrospective study with a highly selected patient group, introducing potential biases that limit the strength of its conclusions. The other was a prospective study of 40 women with eclampsia. One out of 40 developed epilepsy and four out of 40 had new-onset migraine-like headache at follow-up 3-6 months after delivery.[182] They concluded that a minority of patients may develop new

neurological symptoms after a pregnancy complicated by eclampsia. However, their follow-up time was short, only a small proportion of the women were eligible to follow up, and diagnosis of neurological symptoms was solely based on self-reported history.

The pathophysiological mechanisms behind the association between preeclampsia, eclampsia and epilepsy are not established. Women with epilepsy prior to pregnancy face an increased risk of developing severe preeclampsia, HELLP syndrome, or eclampsia during pregnancy or within 42 days postpartum (aOR 1.30, 95% CI 1.19-1.42).[183] It also seems that eclampsia and epilepsy share some common pathways, such as neuroinflammation, where a review of infectious and inflammatory causes of epilepsy stated that prolonged stimulation of inflammatory signals may lead to persisting damage to the BBB, neuronal death and/or hyperexcitability.[184] In addition, preeclampsia, and in particular eclampsia, are associated with impaired cerebral autoregulation,[39] and neuroinflammation.[63] These mechanisms are assumed to contribute to the formation of cerebral vasogenic edema and irreversible subclinical cerebral infarcts.[51,185,186] Cerebral infarctions, or brain scarring, may serve as focal points for future epileptic activity, and thus would help explain the mechanism underlying subsequent epilepsy.

In an attempt to better comprehend the underlying pathophysiology of the association between preeclampsia, eclampsia and epilepsy, one can look at other neuroinflammatory conditions. A retrospective cohort study reported increased risk of developing epilepsy after surviving intensive care unit-requiring sepsis.[187] The aHR was 1.44 (95% CI 1.15–1.80), thus considerably lower than for eclampsia in our study. This might be explained by their much shorter follow-up time of only two years after sepsis. Another reason might be that they had a non-pregnant, mixed sex population. The inflammatory insult of sepsis was presumably also shorter in duration, since it is an acute condition, compared with the prolonged inflammatory state caused by pregnancy, and especially in preeclampsia, which could cause more chronic damage to the BBB and cerebral parenchyma. Increasing evidence of persistent BBB damage in the context of preeclampsia is also available,[58,63,188] and thus, with similar pathophysiological mechanisms, it is not far-fetched to assume that neurological risks would also coincide. A systematic review and meta-analysis evaluated various biomarkers for epilepsy and identified S100B as a valuable biomarker with prognostic value for epilepsy. The study also highlighted the significant role of neuroinflammation in the pathophysiology of epilepsy.[76]

The associations between gestational hypertension and preeclampsia with migraine and headache are novel findings. Conversely, the association of migraine as a risk factor for preeclampsia is well-established, regardless the presence or absence of aura.[189] Women who experienced migraines during the first trimester of pregnancy were associated with increased risk of developing HDP, and women who medicated for their migraine had a slightly higher risk, compared to women without migraine.[190] The mechanisms behind these

associations are elusive, but may possibly be linked to neuroinflammation, endothelial dysfunction affecting the BBB, alterations in cerebral blood flow, and arterial vasospasm.[51,189] The suggested underlying vascular component is also supported by the fact that both have similar cardiovascular long-term risks.[5,110]

The headache outcome is more imprecise, likely encompassing several different headache diagnoses, including migraine. Therefore, it is challenging to draw conclusions about underlying mechanisms.

The prevalence of sleep disorders and mental fatigue was low, the latter so low that it had to be omitted from separate analyses. Since no primary health care diagnoses were available for the study, this could imply that an existing association remain undetected. Follow-up of patients years after eclampsia found that they reported more cognitive failure, compared with healthy parous controls.[122] This also aligns with clinical experience of women with a history of preeclampsia and eclampsia, who often describe difficulties with memory recall, concentration, and experience fatigue after cognitive tasks. These subjective complaints are consistent with findings of long-term cognitive impairment observed after gestational hypertension and preeclampsia.[115,124] To further examine mental fatigue and sleep disorders, primary health care diagnoses would be of interest. Alternatively, other study designs may be more appropriate, such as case-control studies or qualitative research utilizing questionnaires or interviews.

Paper IV

There is no previously published data on sick leave following childbirth in women who suffered from preeclampsia. In a study using sick leave data from the Swedish LISA database, mothers with intercurrent SLE were more likely to have work loss in the first three postpartum years, compared with mothers without SLE.[139] Two years after childbirth, almost one in three women with intercurrent SLE were on sick leave, compared with one in five women who had suffered from preeclampsia during pregnancy in our study. SLE is a chronic autoimmune disease, and there was an even higher total work loss in their study when they added data from disability pensions.

A study examining women postpartum after pregnancies with gestational diabetes mellitus utilized questionnaires assessing quality of life, dietary intake and physical activity. In this study, sick leave was considered as a covariate. The findings indicated that women with a history of gestational diabetes mellitus reported higher levels of sick leave than controls, four years after childbirth.[140] As mentioned, sick leave was not the main outcome, just a covariate, and the study data was retrospectively collected and self-reported, thus potentially affected by recall bias.

The finding of increased risk of neurological disorders after HDP (Paper III), together with reports on cognitive impairment following preeclampsia or eclampsia,[8,124] as well as MRI findings of persistent BBB leakage and other

long-lasting cerebral effects up to several years after preeclampsia could all be connected to the increase in sick leave.[58,113,116] In addition to this, there are psychological effects having gone through a complicated pregnancy and/or birth, and with that challenges of caring for a preterm infant, which could also affect the mother's well-being.[191]

Clinical implications

Most women will experience at least one pregnancy in their lifetime. Preeclampsia affects 3-5% of pregnancies worldwide, significantly contributing to maternal morbidity and mortality.

The increased BBB permeability seen in the human *in vitro* model after exposure to plasma from women with preeclampsia support that the BBB can be impaired in preeclampsia. The disruption of the BBB was more pronounced in late-onset preeclampsia, aligning with the higher incidence of eclampsia reported in late pregnancy.[19] Participants in the first study did not exhibit severe neurological complications or cerebral edema on MRI, nevertheless, their BBB function was impaired. If severely injured, a loss of BBB integrity may lead to cerebral edema, hemorrhage, or seizures. However, even milder BBB damage could be enough to result in these complications if the cerebral autoregulation is simultaneously impaired.[10] This may explain why some women develop eclampsia despite normal blood pressure readings, and why some women with preeclampsia exhibit edema without progressing to eclampsia.

The correlation between increasing concentrations of NfL in plasma with decreasing TEER measurements in the same study population further indicate that a BBB injury may be present even before severe preeclampsia or eclampsia occur. This is also supported by MRI findings of increased diffusion (subclinical edema) in women with eclampsia and preeclampsia, where no signs of vasogenic edema was seen on conventional MRI.[51]

Manifestations of acute cerebral complications of preeclampsia, such as eclampsia and cerebral hemorrhage or edema, are difficult to predict. A circulating biomarker, alone or in combination with other clinical data, could help identify women with preeclampsia at risk of adverse events and improve the prediction of severe cerebral complications in the future. This would allow for more personalized management of women at high risk, including the implementation of aggressive antihypertensive treatment, the administration of neuroprotective therapies like $MgSO_4$, and informed decision-making regarding the timing of delivery.[102] With the available evidence, NfL seems to be the most promising biomarker for BBB alterations and/or neuroaxonal injury in preeclampsia, however, more studies are needed to explore its full potential.

Women with gestational hypertension, preeclampsia, and eclampsia all seem to be at increased risk of developing neurological disorders in the years following childbirth, compared with women with normotensive pregnancies.

The association between preeclampsia and an increased risk of a composite of medium-term neurological outcomes, as well as individual outcomes of migraine and headache, is novel. We could also confirm previous findings of an association with increased risk of epilepsy after preeclampsia and eclampsia in a new population.[118] The increased risk of developing migraines or headaches in women with preterm preeclampsia aligns with existing knowledge that preterm and term preeclampsia differ in their associated risks for both acute complications and long-term morbidity. Specifically, women with preterm preeclampsia generally face a higher risk of adverse outcomes.

These findings provide clinical context to the previously documented radiological evidence of long-lasting cerebral changes after HDP.[51,58] Moreover, they underscore the necessity to recognize the neurological risks that women with HDP may face years after childbirth. Healthcare professionals, including obstetricians, gynecologists, and other providers, should be informed about these risks and inquire about neurological symptoms during follow-up visits with women who have experienced HDP. Educating affected women about their increased risk enables them to monitor for neurological symptoms and seek appropriate care for diagnosis and treatment. This approach facilitates early detection and timely intervention, potentially preventing unnecessary suffering and long-term morbidity. The responsibility to mitigate these risks begins with obstetricians. Early identification of women at risk for HDP during pregnancy, and effective prevention and treatment strategies, is crucial.

Additionally, healthcare professionals should be aware that HDP may increase the risk of higher sick leave rates in the years following childbirth. One in five women who experience preeclampsia and one in four women with eclampsia, took sick leave exceeding 14 days in the second year after their first childbirth, in comparison with one in six women without preeclampsia. The higher frequency of longer sick leave in these women might imply that they experience some residual symptoms after their pregnancy.

Since data was only available for longer sick leave episodes, differences in shorter sick leaves could not be investigated. No diagnoses were linked to the sick leave data, so whether this increased occurrence of sick leave was truly connected to persisting cerebral damage from preeclampsia, related to other complications of pregnancy, or unrelated, remains to find out.

In summary, women with preeclampsia and eclampsia face an increased risk of medium-term neurological disorders and may struggle to maintain full working capacity in the years following childbirth. This underscores the need for targeted postnatal care for this vulnerable group of women.

Conclusions

The papers included in this thesis enhance our understanding of the pathophysiological mechanisms underlying acute cerebral complications associated with preeclampsia, as well as the effects of preeclampsia and eclampsia on women's health in the years following childbirth. Through the papers included in this thesis, the following conclusions can be drawn:

Paper I

Plasma from women with preeclampsia impairs the BBB in an *in vitro* model using the hCMEC/D3 cell line, measured by changes in TEER and permeability to FITC-Dextran. The effect may be mediated by increased activation of VEGFR2.

Paper II

Plasma concentrations of NfL had a negative association with TEER values in an *in vitro* model of the human BBB. This suggests that BBB alterations may cause NfL to leak from the brain and enter the peripheral circulation.

Paper III

Women with gestational hypertension, preeclampsia, and eclampsia seem to run increased risks of suffering from neurological disorders months to years after giving birth. These neurological disorders include migraine, headache, epilepsy, sleep disorder, and mental fatigue. Women who experienced eclampsia in their first pregnancy had the strongest association with a fivefold increased risk for epilepsy. Different pathophysiological mechanisms may underlie these neurological disorders, depending on which hypertensive disorder the woman was exposed to.

Paper IV

Women with preeclampsia had an increased number of sick leave days and increased occurrence of sick leave episodes in the second year postpartum, compared with women without preeclampsia. Women with preterm preeclampsia had the highest number of sick leave days and sick leave episodes. These findings may reflect difficulties in maintaining full working capacity in the years following childbirth after a pregnancy complicated by preeclampsia.

Future perspectives

Although there have been significant advances in the field of cerebral complications of preeclampsia in recent years, the pathophysiological mechanisms behind acute cerebral consequences and their connection to future neurological morbidity are still not fully understood.

The utilization of *in vitro* models based on human brain capillary endothelial cells introduces new opportunities to study the underlying mechanisms of cerebral involvement and BBB dysfunction in preeclampsia. These *in vitro* models can be further enhanced by co-cultivation or by using human cell lines in dynamic models to improve the phenotype of the cells and even better mimic the conditions of the BBB *in vivo*.

Understanding how, where, and why changes occur in the cerebral vasculature and parenchyma is crucial, as these changes can have significant consequences both acutely and in the long term. Continued research on pathophysiological mechanisms may hopefully contribute to the prevention and treatment of these complications in the future.

As of today, there are no clinical signs or symptoms that can reliably predict cerebral complications in preeclampsia. Further research aimed at finding biomarkers which could help detect cerebral complications in preeclampsia is encouraged. NfL is a promising biomarker for cerebral complications in preeclampsia, but more comprehensive research is warranted to fully understand its role. Future studies should also consider including additional biomarkers beyond those examined in this thesis, such as GFAP and matrix metalloproteinases (MMPs), and explore combinations of biomarkers with clinical data. Immunochemical methods, such as ELISA and Simoa, are increasingly available for clinical use, improving detection capabilities of various biomarkers. This advancement suggests that, in the future, if one or more cerebral biomarkers are identified to accurately predict diagnosis or prognosis, they could be utilized to prevent adverse maternal outcomes in women with preeclampsia.

The different subtypes of HDP may have different pathophysiological backgrounds resulting in these outcomes. Gestational hypertension and preeclampsia are associated with similarly elevated risks of cardiovascular morbidity later in life, suggesting a potential vascular component. Eclampsia, however, may cause scars in the cerebral tissue that could serve as a focus for

later epileptic activity. This calls for further studies of the pathophysiological pathways of neurological complications to HDP in the future.

Larger studies, including more women, or other study designs are suggested for future studies in order to increase power for rare diagnoses like eclampsia and HELLP syndrome.

Future investigations into sick leave after preeclampsia would benefit from detailed diagnostic information provided on medical certificates. This information is essential to determine whether sick leave reasons are attributable to complications of preeclampsia. In Sweden, this data is accessible through the Swedish Social Insurance Agency. Studies employing standardized questionnaires, such as cognitive tests and depression scales, can provide a structured approach to examining various health aspects in relation to sick leave. It would also be valuable to consider employment status and occupation in future studies.

Continued translational research regarding preeclampsia and how it affects the brain is needed. By investigating the underlying pathophysiological mechanisms of acute cerebral complications associated with preeclampsia, a better understanding of their connection to long-term consequences can likely be achieved.

Swedish summary (svensk sammanfattning)

Preeklampsi (havandeskapsförgiftning) drabbar 3–5% av gravida kvinnor. Diagnosen ställs vid nydebuterat högt blodtryck (>140/90mmHg) och tecken på organpåverkan efter graviditetsvecka 20. Preeklampsi är en av de vanligaste orsakerna till sjukdom och död hos såväl mor som barn. Vid preeklampsi är hjärnkomplikationer såsom eklampsi (kramper), hjärnödem (svullnad) och hjärnblödningar bland de vanligaste orsakerna till att mamman dör.

De bakomliggande mekanismerna till eklampsi och hjärnpåverkan vid preeklampsi är inte helt klarlagda. Studier tyder dock på att det uppstår en skada i blod-hjärnbarriären med ett ökat läckage av vätska från blodbanan in till hjärnan med hjärnödem som följd. De flesta tidigare studier har huvudsakligen utförts på djurmodeller, då det är både etiskt och tekniskt svårt att studera blod-hjärnbarriären hos gravida kvinnor.

Tidigare betraktades de akuta förändringar som observerades i hjärnan vid preeklampsi som reversibla. Studier har på senare år dock visat på kvarstående förändringar i hjärnan. Bland annat så har magnetkameraundersökningar påvisat läckage över blodhjärnbarriären flera år efter genomgången graviditet komplicerad av preeklampsi.

Kända långsiktiga konsekvenser efter preeklampsi innefattar ökad risk för hjärtkärlsjukdom, kognitiv svikt, stroke och demens. Neurologiska sjukdomar i närmare anslutning till förlossning är dock inte tidigare studerat. Likaså är det okänt om preeklampsi påverkar kvinnans förmåga att återgå i arbete efter graviditeten.

Den övergripande målsättningen med denna avhandling var att studera hur mammans hjärna påverkas av en graviditet komplicerad av preeklampsi.

Studie I och II grundar sig på laboratoriearbeten, där en *in vitro*-modell med mänskliga hjärnendotelceller (celler från hjärnans kärl) användes för att undersöka bakomliggande mekanismer i blod-hjärnbarriären på cellnivå. Detta gjordes genom att studera hur blod från gravida kvinnor med preeklampsi, gravida kvinnor med normalt blodtryck och icke-gravida kvinnor påverkade cellerna i modellen. Blod-hjärnbarriären utgör ett skyddande skikt mellan blodet och hjärnan. Den är uppbyggd av celler i hjärnans kärlvägg (endotelceller) som binds samman tätt av kopplingar mellan dem, samt av stödjeceller och nervceller i hjärnan. Blod-hjärnbarriären är viktig för att upprätthålla en stabil

och säker miljö för hjärnans funktion och hindrar skadliga ämnen från att komma in i hjärnvävnaden.

I den första studien undersöktes blod-hjärnbarriärens funktioner efter tillsats av blod från kvinnorna. Celler odlades i enkla lager i en särskild behållare och mätningar av genomsläpplighet (permeabilitet) och transendotelial elektrisk resistens (TEER) gjordes efter tillsats av plasma från kvinnorna. Celler som behandlats med blod från kvinnor med preeklampsi uppvisade en ökad permeabilitet och minskad TEER jämfört med de celler som behandlats med blod från gravida kvinnor med normalt blodtryck och icke-gravida kvinnor.

I den andra studien undersöktes nivåer av fyra olika hjärnbiomarkörer (NfL, tau, NSE och S100B) i blodet hos kvinnor med preeklampsi, gravida kvinnor med normalt blodtryck och icke-gravida kvinnor. Samtliga av dessa biomarkörer förekom i förhöjda nivåer i blodet hos kvinnor med preeklampsi jämfört med gravida kvinnor med normalt blodtryck. Vidare undersöktes samband mellan förändringar i nivåer av biomarkörer i blodet och TEER-värden i *in vitro*-modellen efter tillsats av blod från kvinnorna. Det konstaterades att högre nivåer av NfL i blodet korrelerade till en större förlust av TEER i *in vitro*-modellen.

I studie III och IV användes svenska register för att utforska hur graviditetsrelaterade blodtryckssjukdomar påverkar kvinnors risk att drabbas av neurologiska sjukdomar och sjukskrivning under åren efter förlossning. Gravida kvinnor identifierades i Medicinska födelseregistret och utfallsvariabler hämtades från Patientregistret avseende neurologiska diagnoser, respektive Longitudinella statistikdatabasen (LISA) för sjukskrivningsdata.

I den tredje studien undersöktes risken att drabbas av neurologiska sjukdomar (migrän, huvudvärk, epilepsi, sömnstörning och hjärntrötthet) åren efter en graviditet komplicerad av graviditetshypertoni, preeklampsi eller eklampsi jämfört med en graviditet med normalt blodtryck. Närmare 650,000 kvinnor som födde sitt första barn mellan 2005 och 2018 inkluderades i studien. Kvinnorna följdes upp avseende eventuell nytillkomst av en neurologisk diagnos från sex veckor efter förlossningen till och med 2019. I den första analysen undersöktes en sammansättning av de fem diagnoserna. Det framkom en högre risk att drabbas av någon neurologisk sjukdom åren efter graviditet om graviditeten komplicerats av graviditetshypertoni, preeklampsi eller eklampsi, jämfört med om man hade en graviditet utan högt blodtryck. Därefter analyserades varje diagnos för sig. Kvinnor med preeklampsi hade ökad risk att drabbas av migrän, huvudvärk och epilepsi åren efter sin första graviditet, den största risken förelåg dock för kvinnor med eklampsi att drabbas av epilepsi.

I den fjärde studien undersöktes eventuella skillnader i sjukskrivningsmönster hos kvinnor med och utan preeklampsi under det andra året efter förlossning. Drygt 95,000 kvinnor som födde sitt första barn mellan 2016 och 2017 inkluderades. Det totala antalet registrerade sjukskrivningsdagar i LISA-databasen (sjukskrivningar överstigande 14 dagar) samt antalet

sjukskrivningstillfällen överstigande 14 dagar under andra kalenderåret efter förlossning analyserades. Initialt undersöktes skillnader mellan kvinnor med eller utan preeklampsi. Kvinnor som drabbades av preeklampsi under sin första graviditet hade både en högre andel sjukskrivningsdagar och fler långtidssjukskrivningstillfällen jämfört med kvinnor utan preeklampsi. Därefter gjordes en indelning av kvinnor med preeklampsi i grupper utifrån komplikationer kopplat till sjukdomen; eklampsi, HELLP-syndrom (påverkan på levern och blodet), eller ingen av dessa komplikationer, samt en indelning utifrån graviditetslängd vid förlossningen; preeklampsi med förtidsbörd (förlossning före 37 fulla graviditetsveckor) respektive förlossning i fullgången tid (förlossning vid eller efter 37 fulla graviditetsveckor). Kvinnor med eklampsi hade högst antal sjukskrivningsdagar, men då det var för få kvinnor med eklampsi i analysen så kunde inte resultatet säkerställas statistiskt. Preeklampsi med förtidsbörd innebar dock en högre risk för ökad sjukskrivning.

Sammanfattningsvis fann vi stöd för att blod-hjärnbarriären påverkas vid preeklampsi genom nedsatt funktion och ökat läckage i en *in vitro*-modell baserad på mänskliga hjärnendotelceller. Huruvida detta ensamt eller i kombination med andra mekanismer, såsom inflammation i hjärnan, förändringar i hjärnans förmåga att reglera blodflödet eller genom kramper i hjärnans kärl, ligger bakom den hjärnpåverkan som kan ses hos kvinnor med preeklampsi och eklampsi återstår att undersöka vidare. Den ökade förekomsten av biomarkörer i blodet vid preeklampsi, och sambandet mellan NfL och TEER, talar för att det vid preeklampsi sker en strukturell påverkan på hjärnan och/eller blod-hjärnbarriären. Detta skulle förutom att ge akuta symptom även kunna orsaka permanenta skador på blod-hjärnbarriären och hjärnan och därmed kunna vara en del av förklaringen till den ökade förekomsten av neurologiska sjukdomar som vi fann hos kvinnor med preeklampsi och eklampsi åren efter graviditet. Att preeklampsi kan orsaka permanenta skador på hjärnan och blod-hjärnbarriären styrks även av de magnetkamerastudier och stora registerstudier som visat att preeklampsi medför en ökad risk för kognitiv svikt, demens och stroke senare i livet. Slutligen fann vi att kvinnor med preeklampsi i högre grad var långtidssjukskrivna än kvinnor som inte drabbats av preeklampsi under graviditeten. Vad denna ökade sjukskrivning står för är inte klarlagt, men det talar för att kvinnor som haft en graviditet komplicerad av preeklampsi tycks ha en ökad sårbarhet även efter graviditeten.

Våra resultat ger underlag för att kvinnor med preeklampsi borde erbjudas en mer strukturerad uppföljning efter graviditeten avseende riskerna för hjärnpåverkan, vilket saknas idag.

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