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Cerebral biomarkers in women with preeclampsia

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Abstract

Bergman, L. 2017. Cerebral biomarkers in women with preeclampsia. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine* 1364. 98 pp. Uppsala: Acta Universitatis Upsaliensis. ISBN 978-91-513-0057-3.

Preeclampsia and eclampsia are among the most common causes of maternal and fetal mortality and morbidity worldwide. There are no reliable means to predict eclampsia or cerebral edema in women with preeclampsia and knowledge of the brain involvement in preeclampsia is still limited. S100B and neuron specific enolase (NSE) are two cerebral biomarkers of glial- and neuronal origin respectively. They are used as predictors for neurological outcome after traumatic brain injuries and cardiac arrest but have not yet been investigated in preeclampsia.

This thesis is based on one longitudinal cohort study of pregnant women (n=469, Paper I and III), one cross sectional study of women with preeclampsia and women with normal pregnancies (n=53 and 58 respectively, Paper II and IV) and one experimental animal study of eclampsia (Paper V).

In Paper I and III, plasma concentrations of S100B and NSE were investigated throughout pregnancy in women developing preeclampsia (n=16) and in women with normal pregnancies (n=36) in a nested case control study. Plasma concentrations were increased in women developing preeclampsia in gestational week 33 and 37 for S100B and in gestational week 37 for NSE compared to women with normal pregnancies.

In Paper II and IV, increased plasma concentrations of S100B and NSE were confirmed among women with preeclampsia compared to women with normal pregnancies. Furthermore, increased plasma concentrations of S100B correlated to visual disturbances among women with preeclampsia (Paper II) and plasma concentrations of S100B and NSE remained increased among women with preeclampsia one year after delivery (Paper IV).

In Paper V, an experimental rat model of preeclampsia and eclampsia demonstrated increased serum concentrations of S100B after seizures in normal pregnancy (n=5) and a tendency towards increased plasma concentrations of S100B in preeclampsia (n=5) compared to normal pregnancy (n=5) without seizures. Furthermore, after seizures, animals with magnesium sulphate treatment demonstrated increased serum concentrations of S100B and NSE compared to no treatment.

In conclusion; plasma concentrations of S100B and NSE are increased in preeclampsia during late pregnancy and postpartum and S100B correlates to visual disturbances in women with preeclampsia. The findings are partly confirmed in an animal model of eclampsia.

Keywords: preeclampsia, eclampsia, biomarkers, S100B, NSE, PRES

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Jag tror
När vi går genom tiden
Att allt det bästa
Inte hänt än

Till Kalle

List of papers

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

I Wikström, AK., **Bergman (maiden name Ekegren), L**., Karlsson, M., Wikström, J., Bergenheim, M., Åkerud, H. (2012) Plasma levels of S100B during pregnancy in women developing pre-eclampsia. *Pregnancy Hypertension*, 2(4):398-402.

II **Bergman, L**., Akhter, T., Wikström, AK., Wikström, J., Naessen, T., Åkerud, H. (2014) Plasma levels of S100B in preeclampsia and association with possible central nervous system effects. *American journal of Hypertension*, 27(8):1105-1111.

III **Bergman, L**., Åkerud, H. (2015) Plasma levels of the cerebral marker neuron specific enolase are elevated in pregnancy in women developing preeclampsia. *Reproductive Sciences*, 23(3):395-400.

IV **Bergman, L**., Åkerud, H., Wikström, AK., Larsson, M., Naessen, T., Akhter, T. (2016) Cerebral biomarkers in women with preeclampsia are still elevated 1 year postpartum. *American Journal of Hypertension,* 29(12): 1374-1379

V **Bergman, L**., Johnson Chapman, A., Tremble S., Åkerud H., Cipolla, M. (2017) Effect of experimental preeclampsia, seizures and $MgSO_4$ treatment in a rat model on serum levels of S100B and neuron specific enolase. *Manuscript.*

Contents

Abbreviations

BBB	Blood brain barrier
BMI	Body mass index
BP	Blood pressure
CBF	Cerebral blood flow
CNS	Central nervous system
CPP	Cerebral perfusion pressure
CSF	Cerebrospinal fluid
CVR	Cerebral vascular resistance
ELISA	Enzyme-linked immunosorbent assay
GFAP	Glial fibrillary acidic protein
$MGSO_4$	Magnesium sulphate
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
NSE	Neuron-specific enolase
NVU	Neurovascular unit
PRES	Posterior reversible encephalopathy syndrome
PTZ	Pentylenetetrazole
RCT	Randomized controlled trial
ROC	Receiver operating characteristic
RUPP	Reduced uteroplacental perfusion pressure
SGA	Small for gestational age
sEng	Soluble endoglin
sFlt-1	Soluble FMS-like tyrosin kinase

Preface

Preeclampsia is defined as hypertension and proteinuria in the second half of pregnancy and is one of the most challenging diseases known within obstetrics. Ever since first described by Hippocrates 2,400 years ago, the disease has puzzled researchers. Even though we have made progress concerning unraveling the pathophysiology of preeclampsia, conflicting theories still exist, and the debate is certainly alive where the focus of the research has changed back and forth over the years.

Preeclampsia is a disease with possible heterogeneous pathophysiology and a range of adverse maternal outcomes such as liver failure, renal failure, eclampsia and cerebral edema. Efforts to better characterize subtypes of preeclampsia may allow for a clearer understanding of the impact of preeclampsia on maternal and neonatal outcomes. Partly due to lack of knowledge of possible underlying pathophysiological mechanisms, there are not yet any reliable predictors in clinical use to target the majority of women who will develop preeclampsia or to target women with preeclampsia that will develop an adverse outcome. There is also no treatment proven efficient except for primary intervention with aspirin for high-risk women but this treatment seems to prevent mainly early onset preeclampsia which is a small proportion of all women with preeclampsia.

This thesis attempts to aid in understanding preeclampsia and its cerebral effects by measuring cerebral biomarkers in women with preeclampsia. The brain is one of the least explored fields in preeclampsia, but cerebral events are among the complications the obstetrician fears most. There are several methodological and ethical concerns in evaluating the brain in women with preeclampsia, and therefore, thus far, there are mostly animal studies describing the brain in regard to preeclampsia and eclampsia. If brain biomarkers prove to be accurate in reflecting the cerebral pathology in women with preeclampsia and eclampsia, their use could not only help women in regard to an individualized treatment but also lead to a better understanding about the pathophysiological process of brain involvement in preeclampsia.

Introduction

Preeclampsia

Definition

Preeclampsia is a pregnancy-specific disorder that is defined as the de-novo development of hypertension and proteinuria after 20 weeks of gestation.[1] Preeclampsia is often defined as early onset or preterm (diagnosis <34 weeks of gestation or delivery <37 weeks of gestation) or late onset or term (diagnosis ≥34 weeks of gestation or delivery ≥37 weeks of gestation),[2] where women with early onset preeclampsia often have a severe placental dysfunction and a child that is intrauterine growth restricted (IUGR).[3] Preeclampsia can also be divided into mild or severe features, where severe features include an end-organ engagement. This is defined by pulmonary edema, cardiac failure, eclampsia or similar.[4] Severe features can also be defined by severe symptoms or clinical signs such as visual disturbances and severe epigastric pain; abnormal liver function or hematological tests; early onset (<34 weeks of gestation), severe hypertension alone (≥160/110 mm Hg) or fetal morbidity, depending on the classification system.[1]

Moving away from the classical definition, with hypertension and proteinuria as cornerstones for the preeclampsia diagnosis, the majority of societies now recognize preeclampsia as hypertension with one additional organ compromise.[2] Proteinuria does not have to be present for the diagnosis and the amount of proteinuria is not a prognostic factor for the severity of the disease.[4] The clinical definition of preeclampsia according to the International Society for the Study of Hypertension in pregnancy (ISSHP) is as follows:

Preeclampsia is defined by gestational hypertension and one or more of the following: New proteinuria OR one/more adverse conditions OR one/more severe complications.[2]

However, it is still recommended to keep proteinuria as a diagnostic criterion within research settings to ensure more specificity around the diagnosis.[2] This is also the case in the two populations for this thesis.

Epidemiology

Preeclampsia complicates 3-5% of all pregnancies.[5] The incidence of eclampsia varies from 2–3/10,000 births in the western world[6, 7] to 16–69/10,000 births in developing countries.[8] About 9-25% of all maternal deaths are associated with hypertensive disorders in pregnancy.[9, 10] In the Nordic countries, the maternal mortality rate is 7.2/100 000 and hypertensive disorders the second most common cause of death.[11] In preeclampsia, the most common causes of death are due to cerebral complications.[12, 13] Maternal mortality rates have decreased in the western world from the 1990s until the beginning of the 21st century[7] but are still high in low income countries where, especially in Africa and Asia, maternal mortality rates are still 100–200 times higher than they are in Europe and North America.[14]

Preeclampsia and eclampsia are also causes of neonatal morbidity and mortality. In low-income countries, 25% of all stillbirths and neonatal deaths are caused by preeclampsia or eclampsia.[15] Compared to preterm births of other causes, infants of mothers with early onset preeclampsia have higher morbidity and mortality, mostly due to the higher proportion of being small for gestational age (SGA).[16] Overall, in pregnancies complicated by eclampsia, the prevalence of perinatal mortality and/or morbidity is 5.6–11.8%.[17]

Pathophysiology

The pathophysiology of preeclampsia is not completely known, but the hypothesis is based on placental dysfunction with or without underlying endothelial injury due to maternal cardiovascular disease. In early preeclampsia, placental dysfunction is thought to be dominating, whereas in late preeclampsia, maternal factors such as diabetes or obesity are thought to have a higher impact.[18, 19]

The defect implantation of the placenta leads to impaired blood flow and defect remodeling of the spiral arteries. This results in the maintenance of higher resistance in the spiral arteries with subsequent intermittent hypoxia in the placenta. Intermittent hypoxia generates reactive oxygen species, leading to placental oxidative stress and placental dysfunction.[20] Maintenance of higher resistance in the spiral arteries can be measured by uterine artery Doppler. Together, this is called the first stage (Figure 1).

The second stage of systemic maternal disease is defined as exaggerated endothelial activation and a generalized inflammatory state.[21] This is promoted by the release of substances from the intervillous space into the maternal circulation that in turn induce the production of inflammatory cytokines.[22] The generalized inflammation leads to an endothelial dysfunction that includes hypertension, defect glomerular filtration and cerebral edema.[21, 23]

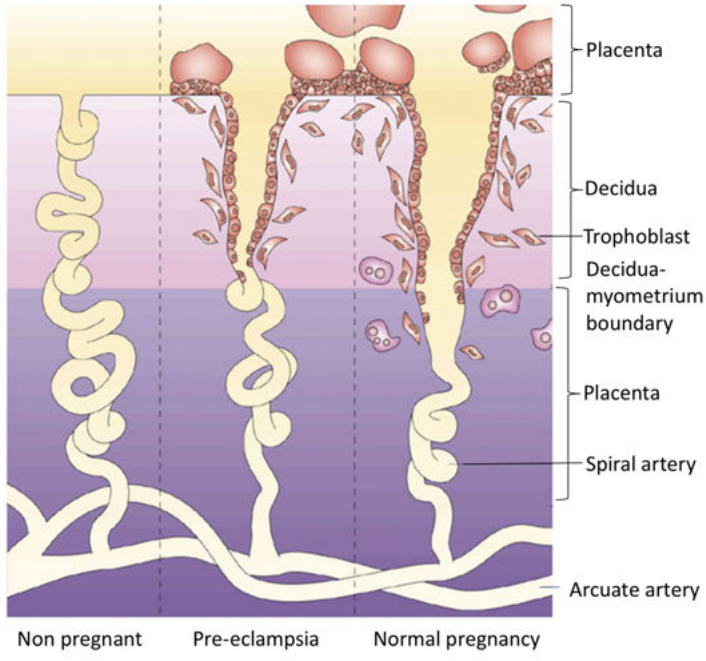

Figure 1. Pathogenesis of preeclampsia. Reproduced from Nature Reviews Nephrology with permission from Nature Publishing Group.

There are different proteins under investigation as contributors to endothelial injury. Of the most thoroughly studied are soluble Fms-like tyrosine kinase 1 (sFlt-1), Placental Growth Factor (PlGF) and soluble Endoglin (sEng). Serum sFlt-1 and sEng are increased in women with preeclampsia many weeks preceding clinical disease, and there is a dose-dependent relationship between serum levels and disease severity.[24] sFlt-1 administered *in vivo* to pregnant rats induces hypertension and proteinuria. Impressively, the co-administration of both sFlt-1 and sEng in pregnant rats recapitulates the entire spectrum of end-organ injury seen in severe preeclampsia.[25] PlGF is lower in women developing preeclampsia and is a promising predictor for early onset preeclampsia in the fist trimester together with blood pressure (BP) and history.[26]

In addition to this supposed pathophysiology, the cardiovascular approach has gained increasingly more interest in recent years. In women with early onset preeclampsia, hemodynamics in mid-gestation are altered with increased vascular resistance, decreased cardiac output, impaired relaxation with mild left diastolic dysfunction and signs of left ventricular remodeling and hypertrophy.[19] Women with this cardiovascular profile in combination with abnormal uterine artery Doppler screening in mid-gestation were more likely to develop early onset preeclampsia compared to women with an abnormal uterine artery

Doppler without hemodynamic alterations. At diagnosis and throughout the postpartum period these changes persist.[19] These findings stress the importance of the cardiovascular remodeling in preeclampsia and the potential use of cardiovascular risk factors for the prediction of preeclampsia and also a window of opportunity for postpartum counseling in these women at higher risk of cardiovascular disease later in life.

Eclampsia

The definition of eclampsia is the onset of generalized tonic-clonic seizures in pregnancies complicated by preeclampsia. The pathogenesis behind eclampsia is not completely understood, but the theories are based on cerebral vasoconstriction or a vasogenic edema predominately in the parieto-occipital regions of the brain.[17] In cases of recurrent seizures there is a higher risk for severe hypoxia, aspiration pneumonia, maternal trauma and status epilepticus.[14, 27] In autopsies of women who have died of eclampsia, there is evidence of intra-cerebral microbleeds, edema and infarctions.[28, 29]

Prediction of eclampsia

In general, screening for a condition among persons at risk should meet certain prerequisites. The condition should be an important health problem, there should be a latent or early symptomatic stage, there should be an effective intervention that results in a better outcome, there should be a suitable test and the benefit should outweigh the potential harm.[30]

No specific prediction model for eclampsia exists. In a systematic review, maternal symptoms (headache, epigastric pain and visual disturbances) could predict adverse maternal or neonatal outcome as a composite outcome but with a poor area under the curve (AUC) of 0.58–0.7.[31] Headache is a common neurological symptom and is present in almost equal percentage in women with preeclampsia with and without eclampsia.[32] There is ongoing research to find a predictive model with or without biomarkers to predict maternal and neonatal complications in women diagnosed with preeclampsia. Studies exploring clinical predictors include the PREP, PIERS and PETRA cohorts and have mainly focused on early onset preeclampsia with populations consisting of 946, 636 and 216 women respectively with complications mainly of sorts other than neurological. The AUC for adverse outcome was about 0.80.[33, 34] A recent study investigating s-Flt-1/PlGF ratio in a low risk cohort of women evaluated the risk of severe preeclampsia at 36 gestational weeks with an AUC of 0.81. In this cohort, there was also a low rate of neurological complications,

where 10/109 women with severe preeclampsia had visual disturbances and no woman had eclampsia.[35]

It is a difficult task to predict eclampsia, and the superior diagnostic tools in today's clinical practice are subjective symptoms. These symptoms may occur before or after the onset of convulsions, and they include persistent occipital or frontal headaches, blurred vision, photophobia, altered mental status, epigastric and/or right upper-quadrant pain. Women who develop eclampsia have at least one of these symptoms in 59–75% of cases.[17]

Eclampsia is not specifically a disease connected to early onset preeclampsia. On the contrary, one-third of eclamptic fits occur antepartum (38–52%), one-third intrapartum (18–35%) and one-third postpartum (11–44%) though eclampsia at lower gestational age has a poorer prognosis for the mother and the fetus.[10, 17] For the intrapartum and postpartum cases, median gestational age is around 38 weeks.[6]

Proteinuria is absent in up to 14% of women when convulsions start, and eclampsia can thus be the first manifestation of preeclampsia.[36] A British study showed that only 38% of women with eclampsia had proteinuria and hypertension the week preceding the eclamptic episode.[7] The occurrence of convulsions is not always correlated to the degree of hypertension. In one study, 16% of women with eclampsia were normotensive at the onset of convulsions.[36]

In summary, no reliable predictor for eclampsia exists and the clinical tools are restricted to subjective symptoms with poor specificity and sensitivity.

Management of preeclampsia and eclampsia

The only evidence based treatments for lowering the incidence of preeclampsia (primary intervention) are aspirin for high risk women (only lowering the incidence of preterm preeclampsia)[26] and calcium supplement, especially in certain populations with low calcium intake.[37]

Since the only definite cure for preeclampsia is delivery of the placenta, problems arise when the woman is diagnosed with preeclampsia preterm. HYPITAT I and II explored expectant management for women with preeclampsia that contributed to current guidelines,[2, 18] where the obstetrician can consider expectant management for women with preeclampsia from gestational age of fetal viability (around gestational weeks 23–24) to gestational week 33 and 6 days. After gestational week 34, the recommendation is to balance toward delivery of women with severe preeclampsia whereas women with preeclampsia

without severe features can be managed expectantly. Signs of severe preeclampsia can be anything from visual disturbances or alterations in liver enzymes to pulmonary edema or renal failure.[38] After gestational week 37, evaluation for delivery should be considered in all cases of preeclampsia.[18, 39]

The cornerstones in the treatment of eclampsia are to prevent maternal injury, support respiratory and cardiovascular function, prevent recurrent convulsions and reduce blood pressure (BP) to a safe range.[17] Systolic BP control is essential in avoiding hemorrhagic stroke and should be kept below 160 mm Hg. [36, 40] One retrospective case study of women with eclampsia showed that 23/24 patients with BP measurements before the stroke event had systolic BP over 160 and 24/24 had a systolic BP over 155 mm Hg. Furthermore, 25/27 (92.6%) were caused by intracerebral bleeding.[40] Stroke can occur in the absence of eclampsia, and it is of great importance to control BP simultaneously when preventing or treating eclampsia. In a retrospective cohort study of stroke during preeclampsia, 0.2% of women with preeclampsia were affected by stroke. Risk factors were black race, older age, severe preeclampsia (42.1% *vs* 29.1%) or eclampsia (28% *vs* 2%). In this study, 47% of preeclampsia-related strokes were bleedings, and the mortality rate for all women with preeclampsia-related stroke was 13.2% compared to 0.02% for women with preeclampsia without stroke. Two thirds of strokes occurred postpartum and out of those, 62% occurred after discharge from the hospital. The degree of hypertension was not evaluated.[41]

In the "Control of Hypertension In Pregnancy" (CHIPS) randomized controlled trial (RCT) for women with gestational or chronic hypertension with either tight control (diastolic BP < 80 mm Hg) or less tight control (diastolic BP < 100 mm Hg), no difference concerning primary or secondary outcomes could be seen (neonatal mortality and morbidity and maternal mortality and morbidity). However, more women with less tight BP control more often developed severe hypertension antenatally.[42]

In summary, the only treatment for preeclampsia and eclampsia is delivery of the placenta and to avoid adverse outcomes such as eclampsia, women are often delivered prematurely where the timing of the delivery relies on symptoms and signs, often of poor predictive value.

Magnesium sulphate

Treatment with magnesium sulphate ($MgSO_4$) is indicated for women with severe preeclampsia for initial stabilization or as treatment peripartum. In high-income countries, this might be revised to women with preeclampsia and

two signs or symptoms of imminent eclampsia.[43] Results from four randomized trials, out of which the Magpie Trial randomizing 10 000 women to treatment with MgSO4 or placebo was the by far largest one,[43] have shown a relative risk of 0.39 and a number needed to treat (NNT) of 71 for developing eclampsia in women with severe preeclampsia. For women with imminent eclampsia (blurred vision, severe epigastric pain or headache) NNT was 36. The incidence of eclampsia in the group that received $MgSO_4$ was 0.6% in contrast to the placebo group, where 2% developed eclampsia. However, the reduction in incidence of eclampsia in the treated group was not associated with a significant benefit in maternal or perinatal outcome. There was also a higher rate of respiratory depression among women treated with $MgSO_4$ in contrast to the placebo group. There is currently no evidence for treatment with $MgSO_4$ for women with preeclampsia without severe features.[27] For women with eclampsia, MgSO4 reduces the risk of recurrent fits and maternal death with 59% and 38% respectively.[17]

Mechanism of $MgSO_4$

The complete mechanism for why treatment with $MgSO_4$ is protective against seizures, and also why $MgSO_4$ is the best treatment available for current seizures in women with eclampsia is not known. Theories include protection of the blood brain barrier (BBB), the vasodilation of cerebral arteries, the reversal of neuro-inflammation and an anticonvulsant mechanism by N-methyl-D-aspartate (NMDA) receptor antagonism.[44-46]

Our research group has conducted a cross-sectional study of women with preeclampsia, women with healthy pregnancies and non-pregnant controls, where all women underwent phosphorus magnetic resonance spectroscopy (MRS) examination of the brain. Compared to women with healthy pregnancies, lower intra-cerebral levels of magnesium were seen among women with preeclampsia as well as a negative correlation between visual disturbances and levels of intra-cerebral magnesium. Serum levels of magnesium did not differ between the groups.[47] This finding indicates that lower intra-cerebral levels of magnesium might be one of the causes of eclampsia in women with preeclampsia.

The action of $MgSO_4$ in preeclampsia and eclampsia has been investigated in rat models with experimental hypertension, preeclampsia and eclampsia. In a rat pregnant hypertensive model where acute hypertension was induced by the infusion of phenylephrine, MgSO4 was shown to reduce BBB permeability with decreased intra-cerebral expression of Evans blue (69 kDa) in animals treated with $MgSO_4$.[48] In the same study, there was no difference in the expression of AQP4 or permeability for sodium flourescein (376 Da) in treated compared to untreated animals. Moreover, there was no difference in brain

water content (see separate heading, *Evaluation of the blood brain barrier*). In a rat preeclampsia model that used the reduced uterine perfusion pressure (RUPP) model (see separate heading, *Animal models in preeclampsia*), animals were allocated to no treatment or infusion with $MgSO_4$. $MgSO_4$ treatment reduced cerebral edema in the anterior brain and reduced protein, albumin/protein and cytokine concentrations in cerebrospinal fluid (CSF) amongst rats with experimental preeclampsia, an effect that was not seen in normal pregnancy.[44]

In a rat model of severe preeclampsia that used RUPP and a high cholesterol diet with induced seizures, treatment with $MgSO_4$ reduced the seizure threshold and degree of neuro-inflammation. The brain water content post-seizure was lower in preeclampsia compared to healthy pregnancy and was not affected by $MgSO_4$ treatment. In preeclampsia, there was an increased concentration of the 470 Da sodium fluorescein stain in preeclampsia, unaffected by $MgSO_4$ treatment, with no difference between healthy pregnancy, preeclampsia and preeclampsia with $MgSO_4$ treatment regarding the larger 70 kDa dextran[45] (see separate heading, *Evaluation of the blood brain barrier)*. These findings were in contrast to earlier studies and could not confirm the BBB protective actions by $MgSO_4$ treatment in this eclampsia model.

In another rat eclampsia model, another mechanism for experimental preeclampsia was used (repeated infusion of lipopolysaccharide [LPS]). Increased brain water content and increased neuro-inflammation were noted in preeclampsia rats with seizures compared to preeclampsia with no seizures. Both neuro-inflammation and brain water content were reduced in preeclampsia with seizures and $MgSO_4$ treatment compared to preeclampsia with seizures without treatment. In addition, rats with preeclampsia and seizures experienced increased concentrations of S100B in CSF and also an increased rate of neuronal death.[49]

In summary, $MgSO_4$ reduces the risk of seizures by 50% but the NNT is high depending on the difficulties to target the population at risk. The mechanism of $MgSO_4$ remains partly unknown.

Cerebral function in pregnancy and preeclampsia

Cerebral hemodynamics during pregnancy and preeclampsia

Cerebral vascular resistance (CVR) and cerebral blood flow (CBF) are determined by vessel caliber and are sensitive to changes in vessel diameter. Cer-

ebral perfusion pressure (CPP) is the difference between mean arterial pressure (MAP) in the circle of Willis and intracranial pressure. In a non-pregnant person, CBF remains at a constant of 50 ml per 100 g of brain tissue when CPP is in the range of 60–160 mm Hg together with a normal intracranial pressure. CBF =CPP/CVR[50, 51]

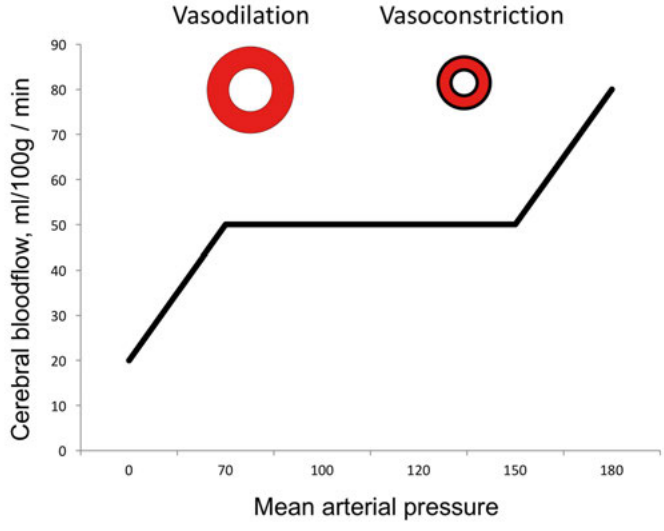

Figure 2. Cerebral autoregulation.

When CPP decreases, myogenic tone decreases, allowing vasodilation to maintain the flow, and, conversely, when CPP increases, myogenic tone increases, leading to vasoconstriction and maintained CBF.[52, 53] CBF is regulated by myogenic, chemical, metabolic and neurogenic mechanisms. The chemical and metabolic functions are controlled by the neurovascular unit (NVU) (see separate section), where chemical signals from neurons, astrocytes and endothelial cells all contribute to the CBF regulation.[54]

Pregnancy is responsible for many hemodynamic changes, including decreased vascular resistance, hyperpermeability and increased cardiac output. Pregnancy alone might remodel the cerebral circulation by a decreased CVR, which leads to a higher hydrostatic pressure and thus increased sensitivity to pressure changes.[55] In animal studies, higher arterial BPs in pregnant rats reduce CVR, increase CBF and enhance BBB permeability compared to non-pregnant rats even in the absence of preeclampsia, whereas there is no difference in BBB permeability in normotensive conditions.[55] There is also evidence that vessels in rats exposed to plasma from women with preeclampsia demonstrate a higher BBB permeability compared to vessels that are exposed to plasma from women with normal pregnancies.[56] Furthermore, in a

preeclampsia rat model, an increased CPP with impaired autoregulatory index and increased brain water content in the frontal lobes were demonstrated in preeclampsia rats compared to normal pregnant rats.[57]

Cerebral autoregulation might be abnormal in women with preeclampsia. In a cross sectional case control study of women with preeclampsia compared to normal pregnant controls, women with preeclampsia had higher CPP and impaired autoregulation of blood flow in response to increased MAP without excessive elevation in MAP over 160 mm Hg. This might explain a possible autoregulatory breakthrough and hyperperfusion without sudden or excessive elevations in BP among women with preeclampsia.[58]

Posterior reversible encephalopathy syndrome (PRES)

Some women with preeclampsia and a majority of women with eclampsia present with an edema on brain magnetic resonance imaging (MRI) in combination with neurological symptoms, a condition known as PRES.[59-62]

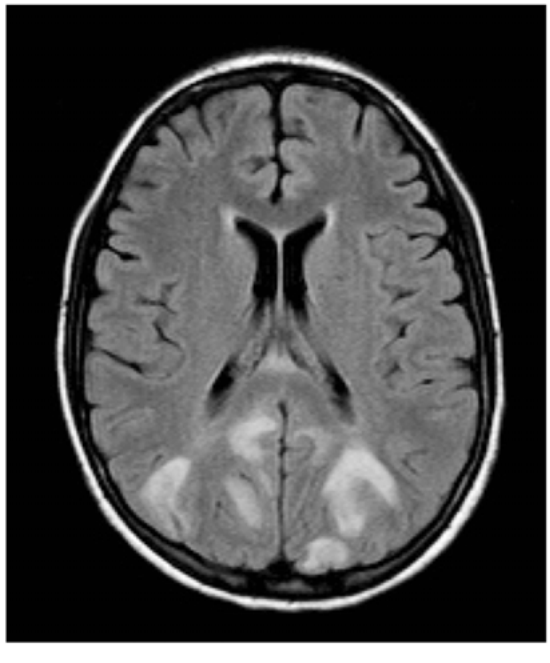

Figure 3. MRI T2 weighted image showing vasogenic edema in the parieto-occipital region. Used with permission from Johan Wikström.

The most commonly affected regions are in the parietal and occipital lobes, supplied by the posterior cerebral artery.[63] However, the distribution is not restricted to these areas and can be found in all subcortical areas of the brain.[54]

The name and definition are sometimes questioned, since the localization and the reversibility of the syndrome is not always true.[54] PRES is not exclusively observed in preeclampsia or eclampsia. Other etiologies include, but are not restricted to, hypertension from other causes,[64] immunosuppressive[65] or cytotoxic drugs[66] and autoimmune conditions.[67]

Current evidence of preeclampsia or eclampsia with PRES is mainly limited to retrospective single-center case studies. The studies are summarized in Table 1. The rate of imaging for eclampsia varied from 38–44%, and, out of these, 59–98% showed signs of PRES. For women with preeclampsia, the percentage of women that underwent imaging was not reported in any of the studies, and in the women undergoing CT or MRI 11–19% showed signs of PRES.[60, 68-72]

Table 1. Five retrospective- and one prospective study of women with eclampsia and preeclampsia that underwent MRI and/or CT scan for evaluation of PRES.

Paper	Eclampsia n (%)	Number (% imaged)	Preeclampsia n (%)	Number (% imaged)	Modality
Brewer, 2013	46/47 (98)	123 (38)	N/A	N/A	CT or MRI
Wen, 2017	26/28 (92)	not reported	7/59 (12)	not reported	CT or MRI
Fisher, 2016	5/8 (63)	not reported	4/38 (11)	Not reported	MRI
Camara, 2017	17/29 (59)	134 (44)	N/A	N/A	CT or MRI
Mayama, 2016	12/13 (92.3)	not reported	5/26 (19.2)	Not reported	MRI
Junewar, 2014	27/35 (77)	35 (100)	N/A	N/A	MRI

Two studies reported on present symptoms; severe hypertension was present in 47–86%, headache in 81–87%, seizures in 72%, altered mental status in 51–53%, visual disturbances in 33–34% and nausea/vomiting in 19–44% of cases.[60, 68] In the study by Wen et al., mean systolic BP was 160 and mean diastolic BP was 100 at the time of imaging.[68] In the study by Fisher et al., seizures were the most common indication for MRI (56%) followed by headache (44%). In this study, no correlation could be seen with symptoms of cerebral involvement, such as visual disturbances, headache or altered mental status with occurrence of PRES.[69] Camara-Lemaroy et al. reported on 44% of cases with eclampsia and PRES to present with neurological symptoms compared to none in the group with eclampsia without PRES. The women with eclampsia and PRES showed signs of more severe disease concerning biochemical characteristics and lower Apgar score.[70] Mayama et al. showed that the preeclampsia and eclampsia group with PRES was similar concerning clinical and radiological findings, supporting the theory of a common pathophysiologic background, and they suggested neuro-radiological imaging for all women with preeclampsia and neurological symptoms.[71]

There is one prospective study of eclampsia and PRES by Junewar et al.[72] Of the 35 women in total, 27 women had PRES, out of which 100% showed vasogenic edema in the posterior and occipital lobes, but frontal lobes were involved in 89% of the cases, reinforcing the notion that PRES is not restricted to the posterior-occipital regions. They also showed that cytotoxic edema was present in 33% of the cases and that altered sensorium, visual disturbances and status epilepticus were associated with presence of PRES but not affected by BP. Serum biomarkers such as lactate dehydrogenase, uric acid and creatinine did not show any correlation to the occurrence or severity of PRES.[72]

The pathogenesis behind PRES is debated, and different theories exist. One is based on loss of autoregulation and subsequent forced dilatation of cerebral arterioles with following edema. The posterior circulation could be more vulnerable due to its lesser innervation from sympathetic nerve fibers.[54] Another is based on rapid fluctuations of BP, where the rapid change is of more importance than the actual BP. Supporting this theory is the fact that only 20% of patients do not have BP in the range over the autoregulatory index.[54] A third theory is based on endothelial dysfunction with or without hypertension, where the endothelial dysfunction leads to the extravasation of fluid through a defect BBB. It is possible that different underlying conditions related to PRES have different pathophysiological mechanisms.[73] There is some evidence that PRES is the actual mechanism behind eclampsia, but most data confirm that it is at least a part of the pathophysiology in eclampsia.[60, 61]

The long-term outcome after PRES due to all underlying pathophysiology was investigated in a retrospective cohort study of 70 patients with PRES, where a good outcome was associated with preeclampsia. Overall, 52.9% of PRES patients had a Glasgow Outcome Score below 5 (bad outcome), whereas the proportion in the preeclampsia/eclampsia group was 5.4% (2/16).[74]

The current gaps in knowledge in cerebral function in preeclampsia are the mechanism that renders women with preeclampsia more vulnerable to cerebral edema, how common PRES is in preeclampsia and eclampsia, how to predict PRES and if cerebral edema is the true underlying cause of eclampsia.

Long-term cerebral outcome after preeclampsia and eclampsia

Women with previous preeclampsia have a two-fold higher risk of developing cerebrovascular disease later in life. [75] The underlying cause could be the common risk factors associated with preeclampsia and cardiovascular disease before pregnancy. However, there are also hypotheses related to the importance

of the cerebrovascular alterations during preeclampsia as a direct cause of the higher risk. This is supported by the fact that endothelial dysfunction in animal models persists after preeclampsia with increased vascular smooth muscle cell proliferation and vessel fibrosis.[76, 77]

In a case-control study of 34 women with previous preeclampsia and 49 women with previous normotensive pregnancies 5–15 years after pregnancy, MRI revealed cortical grey matter reduction via the measurement of total cortical area adjusted for skull volume and increased white matter lesions in women with previous preeclampsia, also after adjusting for confounding factors such as BP and age. In women with previous preeclampsia, the cerebral changes correlated with time from preeclampsia, whereas after normal pregnancy, no such correlation could be seen. There was no difference between women with early onset and late onset preeclampsia.[78]

In another case-control study of 73 women with previous preeclampsia and 79 controls five years after pregnancy, white matter lesions occurred more often and were more severe in women with previous preeclampsia. After adjusting for age, pre-existing hypertension and current hypertension, preeclampsia was still an independent risk factor for white matter lesions. Current hypertension was also a risk factor for white matter lesions in women with previous preeclampsia when adjusting for age. In contrast to the above study, this study showed an increased risk of white matter lesions in women with early onset preeclampsia vs. late onset preeclampsia. Neurological symptoms, $MgSO_4$ treatment or severe hypertension during the preeclampsia episode did not correlate to the presence of white matter lesions.[79] In a cohort study following women with severe preeclampsia from the postpartum period to six months and one year postpartum, white matter lesions were found in 61.7% of women at delivery, 56.4% at six months postpartum and 47.9% at one year postpartum. Lesions were predominately found in the frontal lobes and were positively associated to two or more BP medications during pregnancy and persistent hypertension postpartum.[80]

Long-term sequelae for women with previous PRES during preeclampsia or eclampsia are poorly described and are mainly based on case series and retrospective data. With delayed treatment, PRES might lead to cerebral infarction and hemorrhage, stressing the importance of the early diagnosis and treatment of cerebral involvement in preeclampsia and eclampsia.[81]

Impairment in cognition has also been observed. In a case-control study, 30 women with previous eclampsia and 31 controls were evaluated with a cognitive function test seven years after pregnancy, where the women with pre-

vious eclampsia scored poorer, and the number of fits correlated to a worsening score.[82] In another case-control study, 10 women with previous severe preeclampsia were compared to 10 women with normal pregnancies seven months after delivery. They found that memory was impaired in self-assessed questionnaires in women with previous preeclampsia.[83] A case-control study on the long-term follow up of women with earlier preeclampsia compared to women with previous normal pregnancies used objective cognitive testing and did not find any statistically significant differences but did find a trend towards cognitive impairment in women with preeclampsia with a pattern following the areas of white matter lesions reported in earlier studies.[84] No adjustments for confounders were made, and it is not clear whether these differences also existed before pregnancy. In a recent retrospective study, objective findings of worse performance on tests that processed speed were linked to previous hypertensive disorder in pregnancy even after adjusting for maternal cardiovascular disease.[85]

Preeclampsia has been investigated in the relation to dementia later in life. In a register-based study of 3,232 women, no association between preeclampsia and later dementia could be found (7.6 vs. 7.4%, HR 1.19, 95% CI 0.79–1.73).[86] In a larger register-based study of 248,598 women out of which 505 were diagnosed with preeclampsia during pregnancy, an association between preeclampsia and later vascular dementia was found (HR 6.27, 95% CI 1.65–27.44), but no association could be seen between all hypertensive disorders in pregnancy and vascular dementia or dementia.[87]

In a 2-year follow up of the Magpie Trial, mortality rates were 1% and severe morbidity was 3% among women with previous eclampsia with a mean age around 30 years, stressing the importance of long-term prediction in this young population.[88]

In summary, the reversibility of preeclampsia and eclampsia is now questioned and it seems like women with previous preeclampsia are at higher risk of both cerebrovascular events such as stroke, white matter lesions and vascular dementia and also cognitive failure. It is still unknown whether these events are caused by preeclampsia and eclampsia or if they rather depend on other cardiovascular risk factors already present in these women before onset of pregnancy. There are at present no long-term predictors for neurological outcome.

The blood brain barrier

Structure and function

The BBB is the modified endothelial lining between blood and intra-cerebral tissue. It has a role in maintaining homeostasis (through the regulation of ion balance and compound influx/efflux), protecting the brain from the extra-cerebral environment, supplying nutrients through transport systems and directing inflammatory cells to act in response to change in the local environment.[89] The BBB is more restrictive to the passage of molecules compared to the peripheral vasculature. The anatomic basis of the BBB is composed of a tightly sealed monolayer of brain microvascular endothelial cells characterized by the absence of fenestrations, the low number of pinocytic vesicles and the junctional complex formed by tight junctions and adherent junctions. The properties of the BBB are unique to the brain vascular endothelial cells, and when the vessel diameter increases, the permeability of BBB increases.[90, 91]

The neurovascular unit

Despite the endothelial cells' unique structure, the influence of surrounding microglia, astrocytes, neurons, perivascular pericytes and the basement membrane is important for the overall function of the BBB, and together they form the neurovascular unit (NVU) (Figure 4).

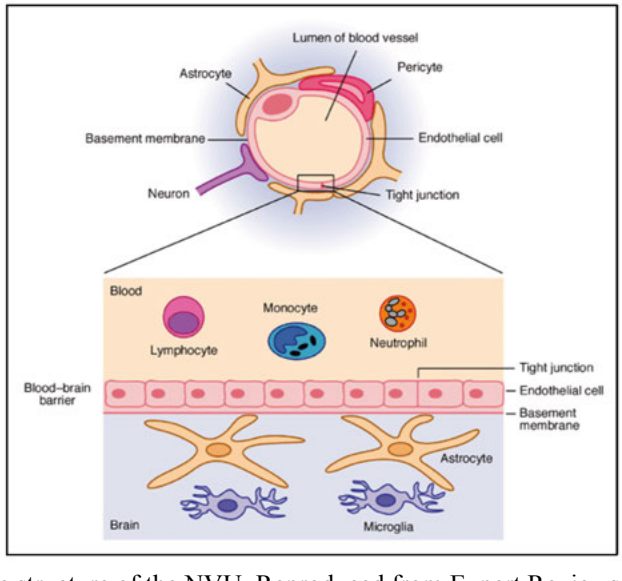

Figure 4. The structure of the NVU. Reproduced from Expert Reviews in Molecular Medicine with permission from Cambridge University Press.

The basement membrane is composed of an extracellular matrix with adhesion receptors and signaling proteins, which are essential for the maintenance of the BBB and are thought to support the cytoskeleton within the endothelial cells. The basement membrane has a negative charge that contributes to the low permeability of the BBB.[92] The digestion of the basement membrane by matrix metalloproteinases results in a disrupted basement membrane and impaired BBB function.[91]

Neurons exert their effect on the BBB and glia cells by direct innervation.[91] Microglia are important for the immune response in the CNS, but their role in the NVU is not clear.[89] Pericytes, or smooth muscle cells, are in close contact with the endothelial cells. They are essential for the structural support and junctional integrity of the BBB and also for blood flow control through their contractile attributes. They also produce an extracellular matrix for the basement membrane, and in traumatic brain injury and/or hypoxia, they migrate away from the BBB, resulting in BBB compromise.[89, 91]

Astrocytes surround the endothelium and basement membrane with their endfeet. They are involved in maintaining the negative charge in the basement membrane that enforces the impermeable structure of the BBB. *In vitro*, culturing endothelial cells together with astrocytes leads to increased transendothelial electric resistance, which resembles the properties of the BBB.[93] Astrocytes also play a major role in neuronal signaling through the BBB.[89]

The endothelial cells form the most important barrier and communicator between blood and brain. The absence of fenestrations, the tight and adherence junctions, the low pinocytic activity and a continuous basement membrane result in a 50–100 times tighter capillary endothelium in the brain compared to the peripheral microvasculature.[89]

Function

In addition to the restrictive function of the BBB, it also has active means of regulation. For example, the brain endothelial cells communicate with astroglial end-feet, where potassium channels enable the active extraction of potassium from the brain to peripheral blood to protect the brain from seizures.[94] In normal conditions, passage through the BBB can occur between cells (paracellular) or through cells (transcellular). Ions and solutes diffuse through the paracellular pathway following their concentration gradient.[95] Lipophilic solutes are allowed to passively diffuse through the paracellular pathwy, but all other substances require active mediated transcytosis or receptor-mediated pathways. The bi-directional transport of hydrophilic molecules, such as peptides and proteins, occurs through BBB transport systems, as the GLUT-1, which transports glucose and other hexoses to the brain.[89]

In an impaired BBB, CBF and vascular tone in the brain are altered, further increasing the transport of molecules and fluid over the BBB.[90]

Evaluation of the blood brain barrier

There are different methods to evaluate the integrity of the BBB. In this section, certain *in vivo* and *in vitro* systems will be covered.

In vivo

One possibility is to evaluate the dynamic aspects of the BBB in real time. This can be done in different ways. One is to calculate the CSF-plasma albumin quotient. In normal conditions, albumin as a protein does not pass the BBB, and a quotient of > 0.007 in humans is considered pathological where a leakage has occurred through the blood-CSF barrier.[96]

There are also different MRI techniques for BBB evaluation. T2 weighted images can give indirect information on BBB dysfunction through the detection of vasogenic edema.[97] An often-used method in clinical practice is to analyze images after the intravenous injection of a gadolinium chelate for areas of enhancement, which is a sign of leakage over the BBB, corresponding to the blood/CSF ratio described above.[98] An example of these contrast agents is gadoliniumdiethylene triamine pentaacetic acid (GD-DTPA), which has a weight of 552 Da and which is much smaller than albumin (68 kDa) that is the molecule used to measure blood/CSF ratio and to evaluate the BBB through immunohistochemistry. There is also a method where GD-DTPA is attached to albumin (Gd-BSA-EB) to render it more comparable to immunohistochemistry studies, but this is used mainly in combined *in vivo* and *in vitro* studies.[99] Leakage over the BBB has been investigated in rat models, where MRI using perfusion techniques with a contrast medium correlated to histological evidence of BBB disruption.[99] These methods have not been investigated in pregnancy.

Another approach to evaluating BBB in animal models is through immunohistochemistry. The mechanism behind all methods within immunohistochemistry is to stain for the presence and intensity of a certain protein. One can choose a protein that is normally not present in the brain tissue around capillaries such as albumin (which is normally only present within the vascular system in the brain). If the staining of albumin occurs outside of the capillaries, this indicates a disrupted BBB.[100] To enhance the sensitivity of the detection of albumin, Evans blue can be used as a tracer, injected before termination.[99] This will detect fairly large defects, since albumin is a 68 kDa protein. One can also trace smaller molecules, such as the sodium fluorescein

molecule (470 kDa).[48] Another possibility is to examine proteins that are expressed within the BBB and investigate whether there is an altered expression of these. Proteins of interest in this method are aquaporin-4 (AQP-4), zonula occludens-1 and glial fibrillary acidic protein (GFAP).[100-102]

Additionally, cerebral edema as a proxy for BBB disruption can be evaluated using brain wet:dry weight after decapitation in animal studies.[45]

In vitro

For an *in vitro* system of BBB to reflect the true state, the cell model must display a restrictive paracellular pathway, possess a realistic cell architecture and display functional expression of transporter mechanisms, and cell cultures should be easy.[103] Cell lines (immortalized brain endothelial cells) are commonly used due to their availability, but primary cultures of brain endothelial cells are preferable due to their higher capability to mimic the true BBB function.[89] Another alternative to human cell lines is brain endothelial cells from animals; these are models that are also widely used, but some caution has to be taken concerning the different properties of human and animal BBB function.[104, 105]A third option is to use pluripotent stem cells that are differentiated into brain endothelial cells.[93] Umbilical vein endothelial cells can be cultured with astrocytes to obtain a BBB-like condition where measurements of transendothelial electric resistance, the permeability of fluorescein isothiocyanate-conjugated dextran and the immunoreactivity of tight junction proteins confirm the attributes of a BBB.[106] More advanced methods where brain endothelial cells are co-cultured with neurons and astrocytes are emerging for a closer resemblance to the BBB *in vivo*.[93]

In the *in vitro* models, cells are arranged in a monolayer, where permeability, electric resistance and expression of tight junction proteins can be evaluated.[106]

The method used for the evaluation of the permeability of BBB *in vitro* is similar to that of immunohistochemistry described in the previous section. With immunoflouresence, it is possible to evaluate permeability to different sized molecules attached to fluorescence tracers, but one should keep in mind that *in vitro* BBB models allow larger molecules to penetrate than do BBB *in vivo*.[89]

In summary, the BBB is challenging to evaluate *in vivo* in pregnant women and most knowledge is derived from *in vitro*- and experimental animal studies.

Cerebral biomarkers

Blood-based and CSF cerebral biomarkers are widely investigated in various neurological disorders, such as traumatic brain injury, neurodegenerative disease and epilepsy. Two of the most promising biomarkers are S100B from glial cells and neuron-specific enolase (NSE) from neurons.[90] Concerning preeclampsia, a few smaller case-control studies have evaluated S100B, but no clinical use for cerebral biomarkers has yet emerged.[107-109] NSE has, to our knowledge, not yet been investigated in preeclampsia.

Ideally, a biomarker should have the following characteristics: 1) represent the pathophysiology behind the condition and be specific for the disorder, 2) appear before the onset of clinical disease, 3) be cheap and easy to measure in blood or urine, 4) display a high sensitivity and specificity for the condition, 5) correlate with the severity of the disease and 6) not be detectable or measured in very low concentrations in normal conditions.[110] For preeclampsia, with the evidence present today, it might be hard to predict all preeclampsia with one biomarker specifically for the disease, since preeclampsia is a multifactorial disease.[111]

The blood brain barrier and peripheral levels of cerebral biomarkers

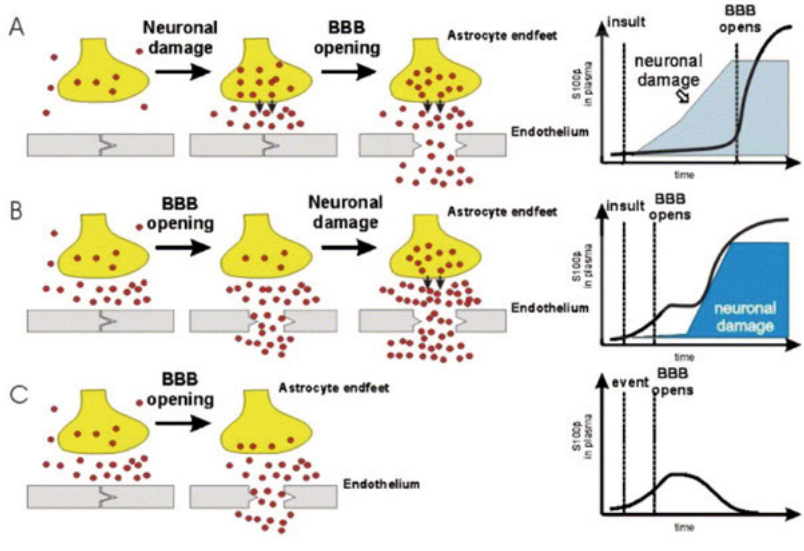

Figure 5. Different options for a biomarker to enter the bloodstream from the CNS, involving an impaired BBB. Reproduced from Clinica Chimica Acta with permission from Elsevier.

Theoretically, several ways exist in which various molecules can pass from blood into cerebral tissue. These include intercellular routes, vesicular transport transcellular or direct transcellular penetration through damaged endothelium.[90] The first two alternatives occur in normal conditions and are well regulated. In these cases, neither S100B nor NSE is transported from the brain into the blood. The damaged endothelium or BBB disruption can either be a causative factor of further BBB disruption or a cause of an intra-cerebral process.[90] Figure 5 illustrates the different means of how a peripheral cerebral biomarker can enter the bloodstream from the brain in combination with BBB disruption.

S100B is normally found in higher concentrations in the CSF compared to peripheral blood, and increased concentrations of plasma S100B is therefore thought to reflect an isolated BBB impairment (Figure 5C). Though, if increased concentrations persist, this could also reflect glial injury alone or in combination with neuronal injury (Figure 5B).[112] The ability of S100B to uniquely reflect BBB disruption without neuronal injury has been supported by studies investigating the effect of osmotic BBB opening without present brain injury where plasma concentrations of S100B were increased.[112]

In contrast, NSE is normally found in higher concentrations in the blood compared to CSF, and the studies above have failed to prove peripheral increased concentrations of NSE in response to an isolated BBB disruption.[112, 113] This implicates that increased peripheral concentrations of NSE suggest a neuronal injury and a compromised BBB (Figure 5A or 5B) rather than an isolated BBB disruption.

To our knowledge, no studies have investigated the CSF concentrations of NSE and S100B in pregnant women, and therefore it is not yet known whether the results from the studies above are generalizable to pregnant women and women with preeclampsia.

S100B

S100B is a protein that was originally purified from bovine brain and was thought to be specific the central nervous system. Subsequent studies showed that S100B consisted of two polypeptides, S100A1 and S100B, most abundant in the nervous system.[114] S100B mRNA is expressed in astrocytes, certain neuronal populations and Schwann cells but also in extra-cerebral cells such as melanocytes, chondrocytes, adipocytes, smooth muscle and heart myofibres and associated satellite cells, some dendritic cells and lymphocyte populations and a few other cell types.[115, 116] In astrocytes, the proteins are synthesized in the end-feet surrounding the BBB and released next to the capillaries

but are secreted into the blood only in the presence of a disrupted BBB.[90] The extra-cerebral concentration is much lower than concentrations in neuronal tissue.[114] S100B forms as a homodimer (BB) or heterodimer (A1B) and has a molecular weight of 8–13 kDa.[114] The homodimer S100BB is almost exclusively present in glial cells.[117] It exhibits intracellular, paracellular and extracellular functions. In low (nanomolar) concentrations, it promotes growth and the differentiation of brain cells, but in higher (micromolar) concentrations, it can exert toxic effects.[118]

The median concentration of S100B in serum among healthy individuals is 0.05 µg/L and is independent of age and gender.[119] Different theories exist as to how S100B is released into peripheral blood, but the most accepted one is that S100B is passively secreted through a compromised BBB after injury.[120] Therefore, elevated concentrations in plasma or serum are not necessarily due to neuronal damage as such.[112] Others debate that it is still unclear whether the release of S100B into peripheral blood requires both irreversible cell damage and BBB disruption or only the latter.[121] S100B is metabolized and excreted through the kidneys, and its half-life is estimated to 30–130 minutes.[122] A cut-off value of 0.10 µg/L has been considered normal, with a slightly higher range among children.[123]

S100B is currently evaluated as a cerebral biomarker for traumatic brain injury. The protein is released during a trauma to the head, and serum levels correlate positively with the degree of cerebral insult induced and negatively with outcome.[124, 125] It has been used as a prognostic biomarker both in minor and severe head injuries and has thus far proven to have best results in the field of minor head injuries. The major benefit is the ability to avoid unnecessary computer tomography scans, and the use of S100B seems to be able to reduce the computer tomography scans by 20–30% (100% sensitivity, 99% specificity and a 99.7 negative predictive value for neurosurgically relevant intracranial complications).[126, 127] It is used in clinical practice in Scandinavia for this purpose in the adult population.[128] In severe head injuries, the potential use of S100B would be to predict outcome and secondary complications. The studies have not been able to concur on a cut-off concentration for S100B, and some studies have shown no correlation between S100B and outcome.[114]

Plasma concentrations of S100B are increased in patients with ischemic stroke[129] and neurodegenerative diseases such as Alzheimer's disease,[117, 130] multiple sclerosis,[131] schizophrenia[132] and bipolar disorder.[133] The mechanisms behind the elevated levels of S100B in these diseases are still poorly understood and need further investigation.

A well-known extra-cerebral source for S100B is malignant melanoma. S100B is used as a biomarker for survival and monitoring in metastatic disease.[134, 135]

Before this project was initiated, a few studies of S100B and preeclampsia had been published. Cai et al. showed that mRNA expression of S100B in placental tissue was higher among women with early onset preeclampsia compared to late onset preeclampsia and healthy pregnant controls.[136] Van Ijsselmuiden et al. examined the intensity of S100B staining through immunohistochemistry of the brains of rats in a preeclampsia model and plasma concentrations of S100B and did not find any difference in staining intensity or plasma concentrations of S100B between groups. In this study, an endotoxin model of preeclampsia was used that models a mild form of preeclampsia. BP elevation, proteinuria or end-organ effects were not reported.[137] Schmidt et al. performed a case-control study in 2004, in which they found elevated plasma concentrations of S100B in women with eclampsia in contrast to healthy pregnant controls, women with gestational hypertension and women with preeclampsia.[108] After the work on this thesis had started, Vetorazzi et al. published a case-control study where they showed that women with severe preeclampsia had higher concentrations of S100B compared to women with mild disease, but they found no correlation to neurological symptoms, though the majority of neurological symptoms constituted of headache.[109] In the beginning of 2015, Artunc-Ulkumen et al. published a case-control study where they found increased concentrations of S100B among women with severe preeclampsia compared to healthy pregnant controls and that higher concentrations of S100B were associated with neurological symptoms including both visual disturbances and headache.[107]

Concentrations of S100B have also been evaluated in umbilical cord blood and have been found in higher concentrations in preterm deliveries; further, S100B protein expression has been found in the placenta and umbilical cord in healthy pregnancies.[138, 139] Neonatal urine concentrations of S100B have been found to correlate to IUGR,[140] and maternal peripheral concentrations of S100B have been shown to correlate to the presence of hypoxic ischemic encephalopathy.[141] None of these studies compared neonatal concentrations to maternal concentrations or adjusted for preeclampsia.

Neuron-specific enolase

NSE is a glycolytic enzyme. It is involved in raising the chloride levels in neurons during the onset of neuronal activity.[121] It is localized in the plasma membrane and in the cytosol.[116] NSE is a member of the enolase family and consists of the γγ homodimer and the αγ heterodimer with a molecular weight of 47 kDa. The mRNA of the γγ homodimer is almost exclusively present in

the cytoplasm of neurons, and the mRNA of the αγ heterodimer is present in diffuse neuroendocrine system (DNES) cells. DNES is a complex system of endocrine cells and nerves corresponding to the epithelial cells and the organ innervation of the respiratory and gastrointestinal tracts.[142-144] Except for neurons and DNES cells, the mRNA of NSE is also present in red blood cells, platelets, endometrial and fallopian tube cells, gallbladder endothelial cells, lung endothelial cells and thyroid, parathyroid and adrenal endothelial cells.[116, 145] The concentration of NSE in the CNS is much higher than in the extra-cranial organs.[116] Since NSE is present in neurons, it is suggested to be a specific neuronal cell marker in contrast to S100B, which is expressed in glial cells.[121, 146] The biological half-life of NSE is probably more than 20 hours.[121]

NSE is a prognostic factor concerning stroke patients, where patients with increased concentrations of NSE had a poorer prognosis at 60 days post-stroke.[129] Plasma concentrations of NSE in relation to prognosis among patients with cardiac arrest with hypoxic ischemic encephalopathy were examined in a large cohort study, where a cut-off of 33 μg/L showed 100% specificity in predicting death or persistent unconsciousness after one month, and NSE in combination with clinical parameters has been recommended as neurologic prognostication in this group of patients.[147] Concerning traumatic brain injury, NSE has been found in increased concentrations in plasma and serum. However, it has shown disappointing results as a possible predictor for outcome compared to S100B. This might be due to its long half-life and also due to its release from red blood cells and platelets.[121]

The protein expression of NSE has been found in the placenta and in the amniotic fluid, where concentrations seem stable with increasing gestational age.[139, 148] Higher concentrations of NSE in amniotic fluid were predictive of neonatal neurologic injury.[149] Median NSE concentration in umbilical cord blood in healthy pregnancies at time of delivery is 8 μg/L.[150] We have found no mRNA studies for the possible origin of NSE protein production in the feto-placental unit.

To our knowledge, thus far no studies have been published on NSE in women with preeclampsia before or during the work on this thesis.

Animal models in preeclampsia

Preeclampsia is predominantly a human disease. There are several animal models to mimic the preeclampsia state, but one must take into consideration that these models do not present the true pathophysiology of the disease.[151] The most commonly used animals are rats, but preeclampsia has also been

modeled in sheep,[152] mice,[153] baboons,[154] rabbits[155] and dogs,[156] amongst others. Rat and mouse models are the best characterized and most used,[157] and in this section, only rat and mouse models will be further described.

Rat and mouse models

Different rat and mouse models of preeclampsia exist. They can be categorized into four different groups, as follows:[157]

1) Animals with surgically induced reduced utero-placental blood flow
2) Animals with preeclampsia-like symptoms induced by pharmacological treatment
3) Genetic animal models
4) Animals with preexisting hypertension developing superimposed preeclampsia during pregnancy

The first model uses reduced blood flow to uterine arteries, the so called RUPP technique. By inducing hypoxia in the placenta, a preeclampsia-like state is achieved with proteinuria, hypertension and end organ damage.[158] This model illustrates both the first placental stage and the second endothelial stage of the disease, where increased peripheral concentrations of sFlt-1 and sEng are found together with a 40% reduction in placental blood flow and 20–30% increase in BP. Furthermore, the model displays endothelial dysfunction and glomerular endotheliosis.[159]

An example of a model induced by pharmacological treatment is the L-NAME model. It is based on treatment with Nω-nitro-L arginine methyl ester that causes the inhibition of nitric oxide synthesis and subsequent vasoconstriction, hypertension and endothelial damage.[160] Another example of a pharmacological model is a technique based on the infusion of lipopolysaccharide (LPS), which induces general inflammation and causes hypertension and proteinuria.[49] Further models include the induction of nephropathy, metabolic models and more recent models with the inhibition of angiogenesis and the activation of the angiotensin type 1 receptor by agonistic auto antibodies.[151] These models can image the endothelial stage of the disease but also to some extent the placental stage depending on at what stage during pregnancy the treatment is initiated.

An example of a transgenic preeclampsia model is the STOX1 mouse model, where the over expression of the STOX1 transcription factor contributes to defect trophoblast invasion with subsequent reactive nitrogen species production, deprivation of maternal nitrous oxide and consequently increased peripheral vascular resistance. The phenotype is similar to the RUPP model and exhibits both placental and endothelial injury.[161]

Preexisting hypertension is achieved by Dahl salt sensitive rats, who present with hypertension and during pregnancy spontaneously develop proteinuria and exacerbated hypertension. In addition, they demonstrate placental hypoxia, reduced fetal growth and increased anti-angiogenic factors.[162] This model is suitable for extrapolation to women with essential hypertension with superimposed preeclampsia.

In the rat model used for this thesis, the RUPP model was combined with a high cholesterol (HC) diet to further enhance inflammatory activity and cause BBB disruption—the RUPP+HC model.[163, 164]

The RUPP+HC model

The mechanism behind the RUPP model is the banding or occlusion of the abdominal aorta inferior to the renal arteries (± banding of the ovarian arteries) or banding the uterine arteries to cause a blood flow reduction of 40–80% to the uterus. The model was originally developed in dogs during the 1940s. The RUPP model for rat emerged in 1987 when Eder and McDonald presented the model consisting of constriction of the infra-renal aorta with silk suture at 14 days' gestation. This has now been developed, and silver clips of set internal diameter placed in the infra-renal aorta and ovarian arteries is the current method of choice.[151] As the hallmark for human preeclampsia is endothelial dysfunction, an animal model must include both hypertension and proteinuria as well as generalized endothelial dysfunction. With the RUPP-model, serum from RUPP rats have been shown to activate endothelium *in vitro* via the angiotensin-1 receptor. RUPP rats also have elevated serum tumor necrosis factor α (TNFα),[165] serum interleukin 6 (IL-6)[166] and serum and placental soluble FMS tyrosine kinase (sFlt-1)[167] and soluble endoglin (s-Eng)[168]—proteins that are also an important part of the pathophysiology in preeclampsia. Therefore, the RUPP model seems to mimic both hypertension and endothelial dysfunction.

An HC diet in pregnant rats is shown to induce endothelial inflammation.[163] To mimic a severe state of preeclampsia in the RUPP+HC model, a high (2%) cholesterol diet is induced from days 7–20 in pregnancy and is combined with the RUPP model to achieve a state of severe preeclampsia with end organ involvement and fetal growth restriction—the RUPP+HC model.[45]

Rationale

Recent research publications are elucidating the brain function in pregnancy and preeclampsia but there are still many gaps in knowledge. It seems like the pregnancy itself remodels the cerebrovascular blood flow and that the endothelial injury and hypertension in preeclampsia lead to BBB injury and brain

edema formation. Though the exact mechanisms why this happens are not yet clear and it is unknown how early in pregnancy this process starts and if it is reversible. Furthermore, there are case control studies that point to persistent brain involvement in women with previous preeclampsia years after the pregnancy. Prediction models of early onset preeclampsia show promising results but adequate prediction of late onset preeclampsia and preeclampsia complications are still lacking. Predictive testing for neurological involvement in preeclampsia is lacking both for short- and long-term outcome. This thesis will not cover all those gaps but by evaluating the role of cerebral biomarkers in preeclampsia we will hopefully gain more insight into the cerebral involvement in preeclampsia that has before mostly been restricted to animal- and experimental studies. Also, by investigating if cerebral biomarkers are increased in preeclampsia we might lead the way to future studies of cerebral biomarkers in preeclampsia as a mean of evaluating women with preeclampsia at risk for cerebral complications at short or long-term.

Overall purpose and specific aims

Overall purpose

The overall purpose of this thesis was to evaluate cerebral biomarkers in women with preeclampsia, during pregnancy and postpartum, and to investigate animal models for cerebral biomarkers in preeclampsia and eclampsia.

Specific aims

I To evaluate whether there is a difference in the plasma concentrations of S100B throughout pregnancy in women who developed preeclampsia and women who did not.

II To compare plasma concentrations of S100B in women with preeclampsia with concentrations in healthy pregnant controls and furthermore to analyze plasma concentrations of S100B in relation to possible CNS effects.

III To compare plasma concentrations of NSE throughout pregnancy in healthy pregnant women and in women who developed preeclampsia.

IV To compare plasma concentrations of NSE and S100B during pregnancy and one year postpartum in women who have had preeclampsia to women with normal pregnancies.

V To determine the effect of experimental preeclampsia and seizures on serum concentrations of S100B and NSE and how treatment with magnesium sulphate influences the concentrations of S100B and NSE.

Material and methods

Overview of the studies

Table 2. Study design, populations, time periods and outcome for the studies.

Paper	Design	Subjects	Period	Outcome
I	Nested case control study from prospective cohort*	16 women developing preeclampsia and 36 healthy pregnant controls	2004-2007	Plasma concentrations of S100B during pregnancy
II	Case control study#	53 women with preeclampsia and 58 healthy pregnant controls	2007-2010	Plasma concentrations of S100B and neurological symptoms
III	Nested case control study from prospective cohort*	16 women developing preeclampsia and 36 women with healthy pregnancies	2004-2007	Plasma concentrations of NSE during pregnancy
IV	Case control study#	53 women with previous preeclampsia and 58 pregnant controls	2007-2010	Plasma concentrations of S100B and NSE during pregnancy and one year postpartum
V	Experimental animal study	Sprague Dawley rats; 5 healthy pregnant, 5 RUPP+HC without and after seizures and 4 RUPP+HC+$MgSO_4$ after seizures	2014	Serum concentrations of S100B and NSE without and after seizures and with $MgSO_4$ treatment

*Paper I and III are derived from the same cohort study population

#Paper II and IV are derived from the same case control study population

Study populations and study design

Papers I and III

A cohort of healthy pregnant women (n = 469) was enrolled in gestational weeks 8–12 at five participating prenatal centers in Värmland, Sweden from autumn 2004 to spring 2007 for the purpose of prediction of preeclampsia. The population is presented in Figure 6. Only women with singleton pregnancies were recruited. Women with a concurrent diagnosis such as chronic hypertension, episodes of high BP before pregnancy, persistently elevated BP before the 20th week of gestation, upper urinary tract infection, pre-existing

renal disease, diabetes mellitus or drug abuse were not included. Plasma samples were collected at the antenatal health care centers at gestational weeks 10, 25, 28, 33 and 37 according to the general controls for antenatal care in Sweden.

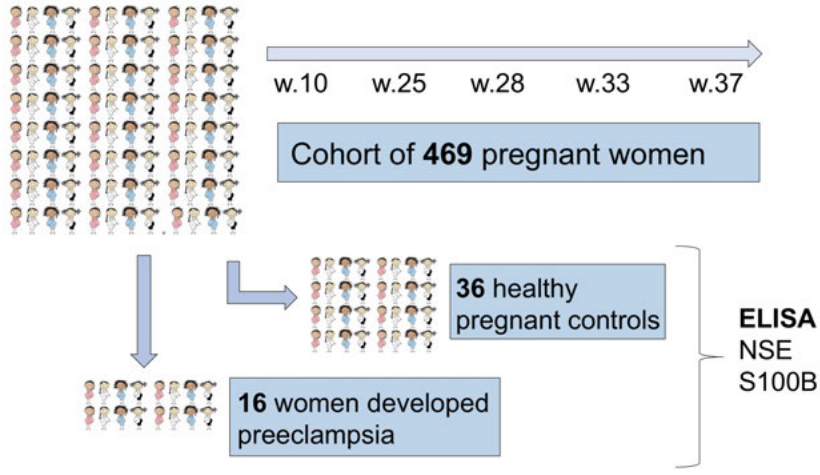

Figure 6. Study population for Paper I and III

Preeclampsia was defined as new-onset hypertension (140/90 mm Hg or greater) observed on at least two separate measurements six hours or more apart combined with proteinuria (two or more on a dipstick or a 24-hour urine sample showing 300 mg/24 h or more). From the cohort 20 women developed preeclampsia, and blood samples were available from 16 women. A total of 302 women in the cohort had a normal healthy pregnancy and delivered at full term (≥ 37 gestational weeks). From these, 36 women were randomly selected and included in the study as controls.

Papers II and IV

This study was designed as a case-control study for the investigation of arterial wall dimensions in preeclampsia. The women were recruited between 2007 and 2010 at the Department of Obstetrics and Gynecology, Uppsala University Hospital, Uppsala. The population is presented in Figure 7.

The cases (n = 53) were pregnant women with preeclampsia. They were included at the time when the diagnosis was confirmed. The definition of preeclampsia was new-onset hypertension (≥ 140/90 mmHg, observed on at least two separate measurements ≥ six hours apart), combined with proteinuria (≥ two units on a dipstick or a 24-hour urine sampling showing ≥ 300 mg

albumin/24 hours) after gestational week 20. The controls (n = 58) were frequency matched according to gestational weeks ± 2 weeks and consisted of women with normal pregnancies defined as an uncomplicated progress throughout the whole pregnancy with delivery after 37 weeks of gestation. Women were not included if they were suffering from chronic hypertension, renal disease or diabetes mellitus or if plasma samples from inclusion or the postpartum examination were not available, since the S100B analysis was then not possible.

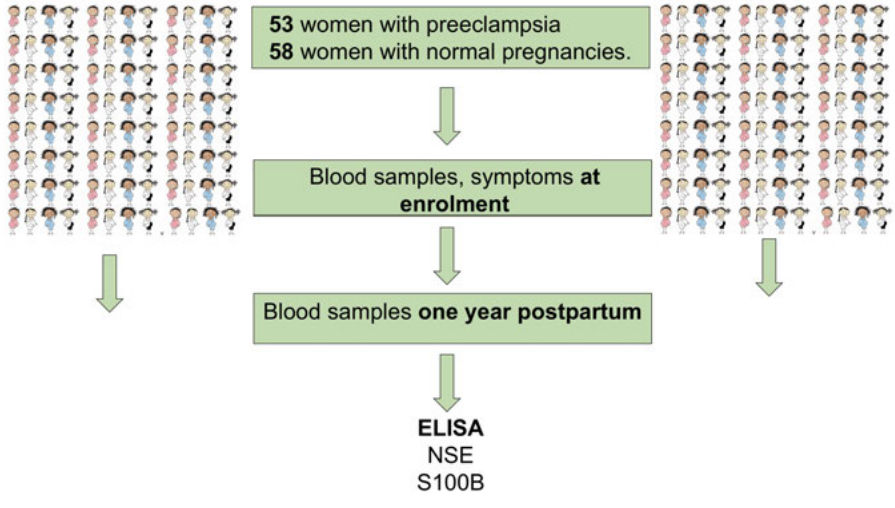

Figure 7. Study population for Paper II and IV

At inclusion, maternal height, weight and BP were registered. Information about reproductive history and smoking habit as well as pregnancy-related complications, gestational age at delivery and birth weight of the infant was collected from the medical records. Gestational age was defined as completed weeks of gestation calculated from the estimated date of delivery according to the early second trimester dating ultrasound.

At the postpartum examination, about one year after delivery, another blood sample was taken, BP was measured and BMI was calculated. Three women in the preeclampsia group were pregnant again, and two did not want to participate. Among the women with normal pregnancies, four women were pregnant. Thus, 48 women in the preeclampsia group and 54 women with normal pregnancies remained in the postpartum evaluation.

Paper V

All experiments were conducted using timed-pregnant Sprague Dawley rats that were 14–16 weeks old. The rats were either normal pregnant or with severe preeclampsia induced by placental ischemia combined with a HC diet (the RUPP+HC model) (n=5/group). One group of experimental preeclampsia rats (n = 4) received $MgSO_4$ prior to experimentation. The experiment was developed and published earlier,[45] and the method is described below in detail. The experimental method is presented in Figure 8.

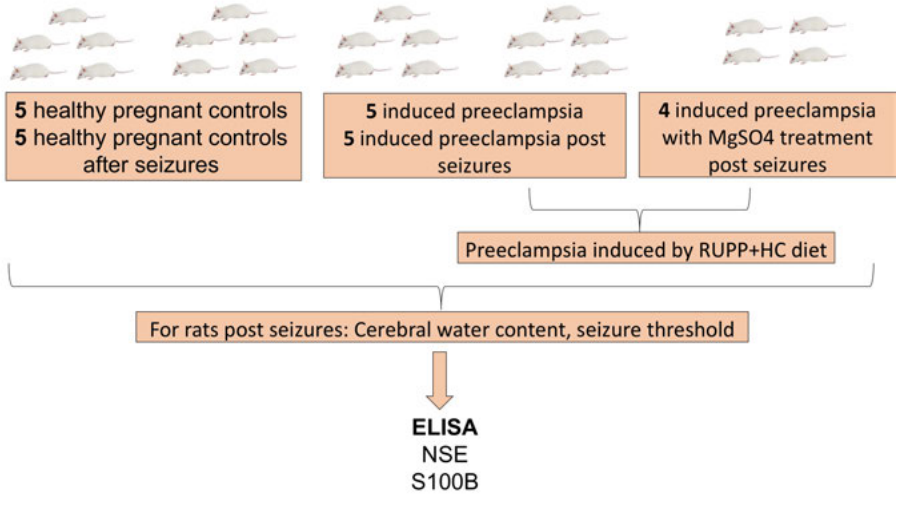

Figure 8. Experimental method for Paper V

On day 14 of pregnancy, a silver clip (diameter 0.203 mm) was placed on the infra-renal aorta, and 0.10 mm silver clips were placed on the arteries of the uterine arcade before the first segmental branch to the utero-placental unit. This reduced the utero-placental perfusion pressure by approximately 40%. Healthy pregnant rats had a sham surgery with the same procedure as the RUPP+HC rats excluding the silver clip placement. All animals were weighed prior to the experiment and euthanized by decapitation after experimentation under anesthesia. Trunk blood was collected and stored in -70 °C until analysis. Uterine horns were examined for number of pups and reabsorbed fetuses.

To establish the model, BP was measured on a separate group of RUPP+HC and normal pregnant animals. Under isoflurane anesthesia, indwelling carotid catheters were implanted on day 18 of gestation. On day 19 of pregnancy, the rats were lightly anesthetized with 1–2% isoflurane for 3–4 minutes and a pressure transducer (BIOPAC, Inc., Goleta, CA, USA) was connected to the indwelling catheter. The rats were placed in a rectangular, clear plastic modular chamber (10×67×65”) with a metal grid floor, large enough to move

freely. Average conscious, unrestrained systolic BPs were recorded using AcqKnowledge software (BIOPAC, Inc., Goleta, CA, USA).

Four RUPP+HC rats were injected subcutaneously on the morning of day 19 of pregnancy with 270 mg/kg 1.0 M MgSO4 (RUPP+HC+MgSO4). Four hours later, the rats were briefly anesthetized with 2% isoflurane and three two-mL osmotic mini-pumps (Alzet, Cupertino, CA, USA) primed with 1.0 M MgSO4 were implanted subcutaneously between the shoulder blades. On day 20 of pregnancy, 1.5 hours prior to surgery and experimentation, the rats were injected with a second bolus of 270 mg/kg 1.0 M MgSO4 subcutaneously.

Normal pregnant rats (n = 5), RUPP+HC rats (n = 5) and RUPP+HC+MgSO4 rats (n = 4) were anesthetized initially with isoflurane (1–3% in oxygen) for intubation, electrode placement and instrumentation. The animals were mechanically ventilated to maintain blood gases and pH within normal physiological ranges. Body temperature was monitored with a rectal thermometer and maintained with a heating pad at 37 °C throughout the experiment. The dorsal surface of the head was shaved to expose the scalp, and silver subdermal corkscrew electrodes (Ambu, Glen Burnie, MD, USA) were implanted under the scalp and secured in place with collodion glue. Electroencephalography (EEG) was recorded in a unipolar mode using a MP150 acquisition system (BIOPAC System Inc., Goleta, CA, USA) to monitor generalized seizures. The recording electrode was placed over the right parieto-occipital cortex (5±0.16 mm lateral and 7±0.16 mm posterior to bregma),[169] a reference electrode was placed in the soft tissue of the snout and a ground electrode was placed posterior to the right ear. Signals were amplified and filtered (low frequency filter, 0.1 Hz; high frequency filter 35.0 Hz) and sampled at 1.0 kHz. After the placement of the electrodes, the animal was placed in a supine position for the placement of venous and arterial catheters. Femoral arteries were cannulated to obtain blood samples for blood gas measurements and continuous measurement of arterial BP via a pressure transducer (BIOPAC Systems Inc., Goleta, CA, USA). As the placement of a silver clip on the distal aorta in the rats with RUPP made monitoring BP in the femoral artery inaccurate, BPs were measured in the axillary artery, as done previously.[170] Femoral veins were cannulated for administration of the anesthetic chloral hydrate and infusion of the chemoconvulsant pentylenetetrazole (PTZ). PTZ was chosen because it reliably elicits seizures by its antagonistic action at the main inhibitory receptors in the brain, gamma-aminobutyric acid (GABA) type A receptors.[171] After instrumentation, the animals were tapered off the isoflurane and anesthesia was maintained by continuous intravenous infusion of chloral hydrate (50 mg/mL; 30 mL/min). Chloral hydrate was used, because it is thought to not depress neural function and is the preferred anesthetic for studies measuring EEG.[172, 173] Seizures were induced by a timed infusion of PTZ (10 mg/mL;

1 mL/min), which was stopped at the first onset of spikewave discharges. The seizure threshold was calculated as the amount of PTZ (mg/kg) required to elicit electrical seizures: T-infusion × R-infusion × [PTZ]/BW, where T-infusion is the time of infusion in minutes, R-infusion is the rate of infusion in mL/min, [PTZ] is the concentration of PTZ in mg/mL and BW is the body weight in kg. Baseline BPs were taken 30 seconds prior to PTZ infusion and at seizure onset. EEG was recorded for 30 minutes post-PTZ infusion, and seizure severity was assessed by counting the number of recurring seizures and calculating the percent of the post-infusion period spent in seizure. After 30 minutes the animals were euthanized under chloral hydrate anesthesia by decapitation and the brains were immediately removed. Serum was collected and stored at -80 °C until use.

The posterior cerebral cortex was isolated and weighed wet, then dried in a laboratory oven at 90 °C for 24 hours and re-weighed dry. Percent water content was determined by wet:dry weights using the following formula: (weightwet – weightdry / weightwet) ×100. The posterior cortex was chosen for measurements, as this is a primary brain region affected in women with preeclampsia and eclampsia.[62]

Statistical methods

Papers I and III

Demographic and clinical characteristics were compared between cases and controls via the Student's t-test and chi-square tests. Plasma concentrations of S100B and NSE were compared between cases and controls using a Mann–Whitney U-test. Values were presented as mean ± SD and median with upper and lower quartiles, respectively. All significance tests were two-tailed. Logistic regression analyses were used to calculate unadjusted and adjusted odds ratio (OR) for the association between preeclampsia and levels of NSE. Confounders were chosen from a pathophysiological point of view and restricted in numbers since the case group only consisted of 16 women. Changes in NSE and S100B concentrations between weeks 10 and 37 within cases and controls were analyzed by the Wilcoxon signed ranked test. The level of significance was set to $p < 0.05$. Statistical analysis was performed using SPSS, version 20.0 (SPSS Inc. PASW statistics) for the MAC software package.

Papers II and IV

Demographic and clinical characteristics were compared between cases and controls via the Student's t-test and chi-square tests. Plasma concentrations of S100B and NSE were compared between cases and controls using a Mann–Whitney U-test. Values were presented as mean ± SD and median with upper and lower quartiles, respectively. Logistic regression analyses were used to calculate unadjusted and adjusted OR for the association between preeclampsia and concentrations of S100B among women with preeclampsia and to calculate unadjusted and adjusted OR for the association between preeclampsia and levels of S100B and NSE one year postpartum. Confounders were chosen according to known extracerebral sources for S100B and NSE. A receiver operating characteristic (ROC) curve was used to determine the ideal cut off for plasma concentrations of S100B and visual disturbances among women with preeclampsia and a Pearson phi correlation coefficient analysis was used to determine if there was a correlation between visual neurological symptoms and plasma concentrations of S100B in preeclampsia. The level of significance was $p < 0.05$. Statistical analysis was performed using SPSS, version 20.0 (SPSS Inc. PASW statistics) for the MAC software package.

Paper V

Serum concentrations of S100B and NSE are presented as medians with SD. Differences in serum concentrations of S100B and NSE between rats with experimental preeclampsia and pregnant controls and within groups with or without seizures were analyzed by a Mann–Whitney U-test. Differences in levels post-seizure were determined by a Kruskal–Wallis test with Dunn's post-hoc test. Correlations between brain water content and seizure threshold with concentrations of S100B and NSE were determined by Spearman's rho. Values are presented as correlation coefficients and p-values. Differences were considered statistically significant at $p < 0.05$. Statistical analysis was performed using SPSS, version 23.0 (SPSS Inc. PASW statistics) for the MAC software package.

Laboratory methods

Enzyme-linked immunosorbent assay (ELISA)

ELISA is a method in which antigens in the specimen attach to pre-coated antibodies to the antigen on the walls of the wells in the ELISA plate, and then another specific antibody is added with an enzyme linked to it. When the enzyme's substrate is added, a reaction permits a signal to be detected, most

commonly a color change. This color change is then translated into a concentration.

Plasma samples were analyzed for concentrations of S100B and NSE using ELISA according to the manufacturer's instructions. For S100B Sangtec 100 ELISA, Diasorin, MN, USA was used. The intra- and inter-assay coefficients of variation were 7.7% and 3.3%, respectively. The limit of detection was 0.015 ug/L. For NSE, DENL20 Neuron Specific Enolase Quantikine ELISA, R&D Systems MN, USA was used. The calibration range was 0.31–20 µg/L. The limit of detection was 0.04 µg/L, and the intra- and inter-assay coefficient of variation was 2.8% and 4.3%, respectively.

For Paper V, commercially available ELISA kits validated for rat serum were used to analyze serum concentrations of both NSE and S100B (Cloud-Clone Corp, Houston, TX, USA). For both NSE and S100B, the intra- and inter-assay coefficients of variation were less than 10% and less than 12%, respectively. For NSE, the detection range was 0.31–20 µg/L. For S100B, the detection range was 0.03–2 µg/L. The samples for the S100B ELISA were diluted 1:3 with 0.01M PBS pH 7. The samples for NSE were not diluted.

Ethical considerations

Informed consent was obtained from all women participating in the studies. The regional Ethics Committee at Uppsala University approved the studies for Papers I–IV. For Paper V, the Institutional Animal Care and Use Committee at the University of Vermont approved the study, and the study was conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. In all experiments, institutional protocols with animals were performed to minimize suffering and limit the number of animals used. The rats were housed in pairs in the University of Vermont Animal Care Facility (an association for the assessment and accreditation of laboratory animal care, an international accredited facility).

Summary of results

Paper I

Maternal characteristics concerning demographic data are presented in Table 3. We did not detect any statistical significant differences between groups in terms of age, parity, BMI, smoking habit, BP at enrolment or infant birth weight. BP at term and gestational age at delivery differed as expected between groups, where women who had developed preeclampsia had higher BP at delivery. A total of 38% of women who had developed preeclampsia had antihypertensive treatment. The median gestational age for diagnosis was 38 weeks. One woman had an abruption placentae and was delivered at 34 weeks' gestation; the others were classified as preeclampsia without severe features.

Table 3. Background characteristics for the population.

	Controls (n = 36)	**Preeclampsia (n = 16)**
Maternal age (years)	30 ± 4	29 ± 4
Nullipara n (%)	21 (51)	13 (59)
Smokers n (%)	0	0
BMI in first trimester (kg/m^2)	24 ± 4	25 ± 5
BP in first trimester (mmHg)		
Systolic	111 ± 10	115 ± 11
Diastolic	65 ± 8	70 ± 9
MAP	80 ± 8	84 ± 8
At delivery		
Gestational length at delivery (weeks)	40.5 ± 6	39.0 ± 10
BP (mm Hg)		
Systolic	122 ± 14	150 ± 16
Diastolic	72 ± 10	98 ± 7
MAP	89 ± 10	116 ± 9
Infant birth weight (g)	3714 ± 375	3587 ± 891

Values are presented as mean ± SD and categorical values as numbers (percentage); Student's t-test; n, number; BMI, body mass index in early pregnancy; BP, blood pressure; MAP, mean arterial pressure

In gestational week 33, there were samples available from 30 healthy controls and eight women with preeclampsia. In week 37, there were samples available

from 26 healthy controls and 10 women with preeclampsia. The plasma concentrations of S100B did not change between gestational weeks 10 and 37 (0.047 vs. 0.052; $p = 0.71$) in the healthy controls, but the concentrations of S100B increased between corresponding weeks in women who developed preeclampsia (0.052 vs. 0.075; $p < 0.05$). In gestational weeks 33 and 37, women who developed preeclampsia had higher concentrations of S100B than the controls ($p = 0.047$ and $p = 0.010$, respectively). The results are demonstrated in the graph in Figure 9.

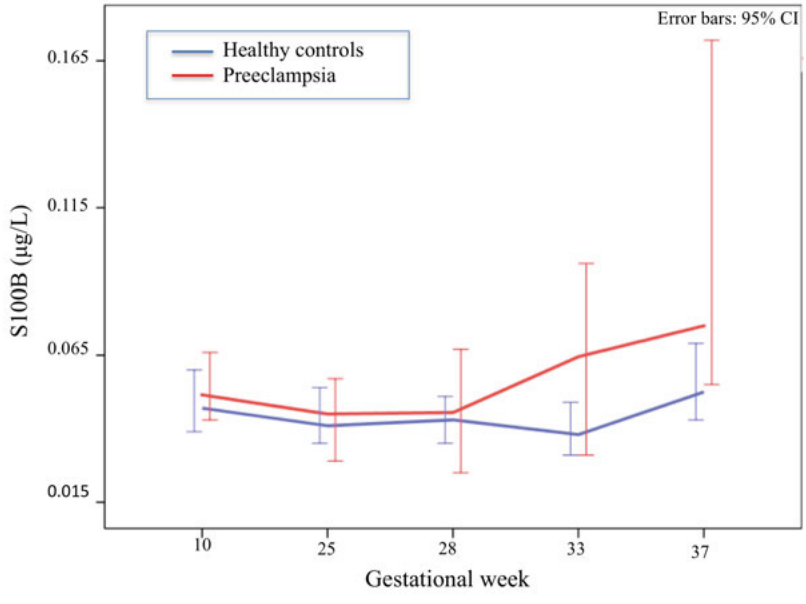

Figure 9. Plasma concentrations of S100B (median) during pregnancy in healthy pregnant controls and women who developed preeclampsia. There was a difference between groups in week 33 and 37. ($p<0.05$)

Paper II

Maternal characteristics and pregnancy outcomes of cases and controls are presented in Table 4. At the first antenatal visit, there were no statistical significant differences between women who developed preeclampsia and controls in terms of maternal age or smoking habit, but they were more often nulliparous and had higher BMI and BP. At delivery, 85% of the women with preeclampsia were treated with antihypertensive medication. Cases and controls were matched for gestational age at time of inclusion, but women with preeclampsia had, as expected, a shorter gestational length at delivery and delivered infants with lower birth weight than controls.

Table 4. Background characteristics for the study population.

	Normal pregnancy (n = 58)	**Preeclampsia (n = 53)**
Maternal age (years)	30 ± 4	30 ± 5
Nulliparous, n (%)	29 (50)	37 (70)
At first antenatal visit		
Smokers, n (%)	2 (3)	0 (0)
BMI(kg/m^2)	23 ± 3	27 ± 6
Systolic BP (mmHg)	114 ± 9	124 ± 11
Diastolic BP (mmHg)	65 ± 7	79 ± 8
MAP (mmHg)	81 ± 7	94 ± 8
At inclusion		
Gestational age (days)	242 ± 34	247 ± 31
BMI (kg/m^2)	27 ± 4	33 ± 7
Systolic BP (mmHg)	114 ± 9	146 ± 12
Diastolic BP (mmHg)	69 ± 7	91 ± 11
MAP (mmHg)	84 ± 7	109 ± 10
Proteinuria*, n (%)	0 (0)	53 (100)
At partus		
Gestational age (days)	280 ± 9	250 ± 29
Antihypertensive drugs, n (%)	0 (0)	45 (85)
Infant birth weight (g)	3658 ± 434	2554 ± 988

Values are presented as mean ± SD; Student's t-test; n, number
*> 2 units on a dipstick

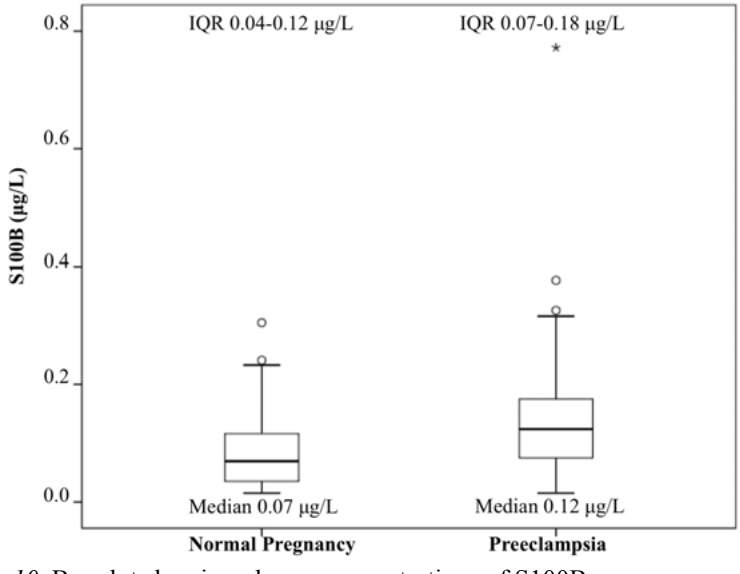

Figure 10. Boxplot showing plasma concentrations of S100B among cases and controls. ($p < 0.001$)

At inclusion, median plasma concentrations of S100B were significantly higher among women with preeclampsia (0.12 μg/L, range 0.03–0.77) compared to controls (0.07 μg/L, range 0.01–0.31) and are further presented in Figure 10 ($p < 0.001$).

A receiver operating characteristic (ROC) curve for S100B and visual disturbances was performed and an optimal cut off of 0.138 μg/L with sensitivity 83% and specificity 38% for visual disturbances was used when determining the correlation between visual disturbances and plasma concentrations of S100B. Six women with preeclampsia had visual disturbances ± 2 days of the time of sampling and there was a correlation between increased concentrations of S100B and presence of visual disturbances ($p < 0.05$).

This association was furthermore analyzed in a regression model, adjusting for headache where visual disturbances was not significantly associated with plasma concentrations of S100B (aOR 8.58, 95% CI 0.90-81.72).

Paper III

The population was the same as the population in Paper I, and background characteristics are presented in Table 3.

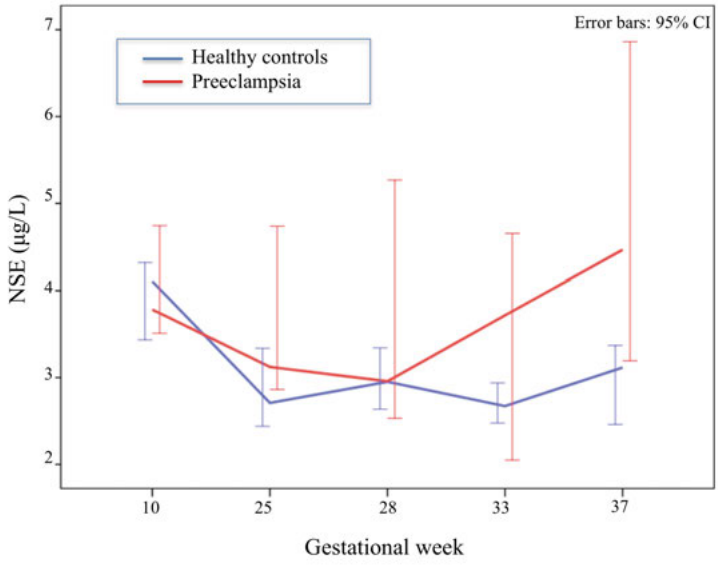

Figure 11. Plasma concentrations of NSE (median) during pregnancy in healthy pregnant controls and women who developed preeclampsia. There was a difference between groups in week 37.

In gestational week 33, there were samples available from 31 healthy controls and eight women with preeclampsia. In week 37, there were samples available from 27 healthy controls and 10 women with preeclampsia. In gestational week 37, women who developed preeclampsia had significantly higher plasma concentrations of NSE than healthy pregnant controls ($p < 0.001$). The plasma concentrations of NSE did not change between gestational week 10 and 37 in women who developed preeclampsia, but the concentrations decreased significantly in healthy pregnant controls ($p < 0.001$). The results are presented in Figure 11. In a multivariable logistic regression analysis where gestational age at delivery and lactate dehydrogenase (LD) as a proxy for hemolysis were added, NSE still remained an independent factor associated with preeclampsia in gestational week 37 (Adjusted OR = 2.29, 95% CI 1.05-4.96).

Paper IV

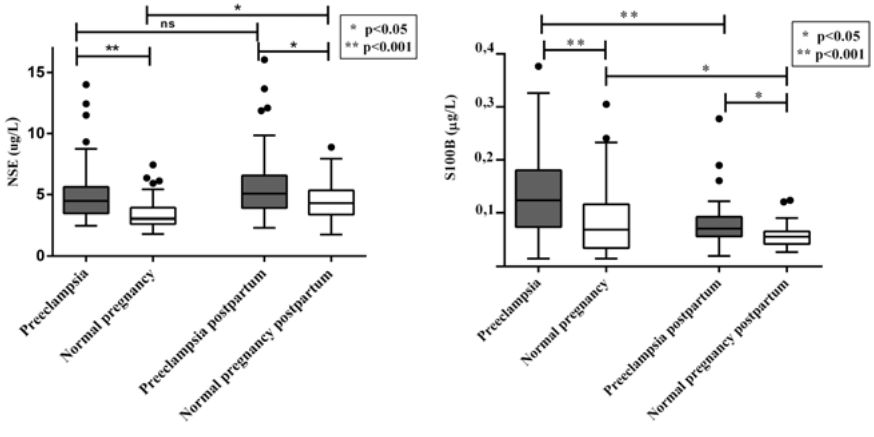

Figure 12. Plasma concentrations of NSE and S100B during late pregnancy and one year postpartum in women with preeclampsia and normal pregnancy.

Median plasma concentrations of NSE during pregnancy in women with preeclampsia were increased compared to women with normal pregnancies (4.47 vs. 3.04 ug/L; $p < 0.001$). Among women with preeclampsia, one year postpartum, the median plasma concentration of NSE was at a level similar to that during pregnancy (5.07 vs. 4.47 ug/L; $p = 0.25$). In women with normal pregnancies, plasma concentrations of NSE were higher one year postpartum compared to concentrations during pregnancy (medians 4.28 vs. 3.04 ug/L; $p < 0.05$). One year postpartum, women who have had preeclampsia still had increased concentrations of NSE compared to women with previous normal pregnancies (medians 5.07 vs. 4.28 ug/L; $p < 0.05$) (Figure 12). When adjust-

ing for common confounding factors—age, parity, BMI and days since delivery—increased concentrations of NSE one year postpartum were still independently associated with previous preeclampsia ($p < 0.01$)

One year postpartum, median concentrations of S100B had declined among women who have had preeclampsia (0.12 vs. 0.07 ug/L; $p < 0.001$). In women with normal pregnancies, concentrations of S100B had also decreased one year postpartum but to a lesser extent (medians 0.07 vs. 0.06 ug/L; $p < 0.05$). One year postpartum, women with previous preeclampsia still had increased concentrations of S100B in contrast to women with previous normal pregnancies (medians 0.07 vs. 0.06 ug/L, $p < 0.05$) (Figure 12). When adjusting for common confounding factors—age, parity, BMI and days since delivery—increased concentrations of S100B at one year postpartum were still independently associated with previous preeclampsia ($p < 0.01$).

Paper V

Maternal body weights and pup and placental weights from RUPP+HC rats were significantly less on day 20 of gestation compared to normal pregnant rats. Further, RUPP+HC rats had increased systolic BP compared to normal pregnant rats. The background characteristics have previously been published.[45]

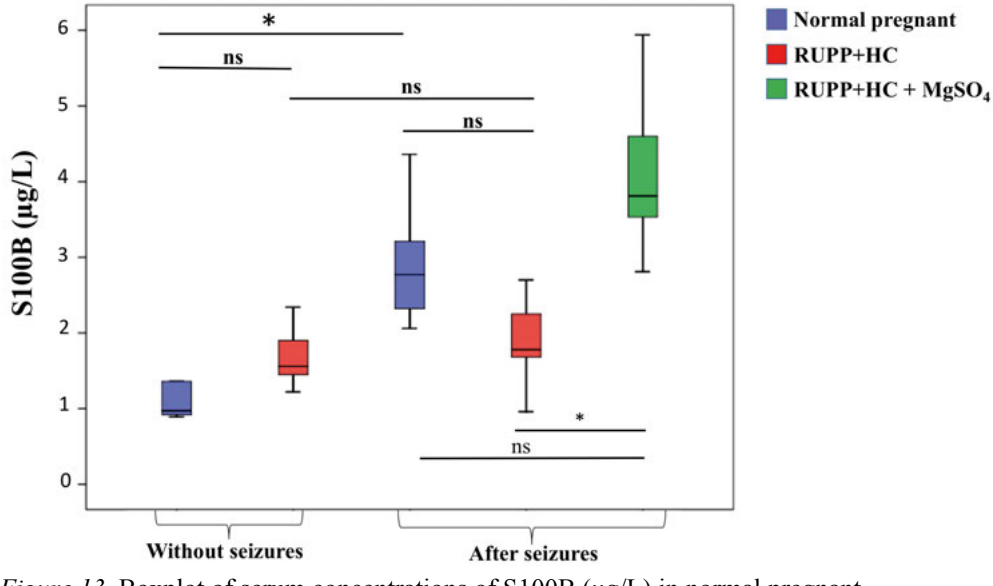

Figure 13. Boxplot of serum concentrations of S100B (µg/L) in normal pregnant rats, RUPP+HC rats and RUPP+HC+$MgSO_4$ rats respectively.

RUPP+HC rats had increased serum concentrations of S100B compared to normal pregnancy, although the difference did not reach statistical significance (1.56 ± 0.44 vs. 0.97 ± 0.58 μg/L, p = 0.12). Serum concentrations of S100B in normal pregnant rats were significantly increased post-seizure compared to normal pregnant rats that did not have seizures (2.77 ± 0.9 vs. 0.97 ± 0.58 μg/L, p < 0.05). Seizures did not affect concentrations of S100B in RUPP+HC rats, indicated by similar concentrations of S100B between RUPP+HC rats with and without seizures (1.78 ± 0.65 vs. 1.56 ± 0.44 μg/L, p = 0.60). In groups that had seizures, there was an increase in serum concentrations of S100B in RUPP+HC+$MgSO_4$ rats and normal pregnancy compared to RUPP+HC rats (p < 0.05). After correcting for multiple testing between groups, the significant increased concentrations that remained were in the RUPP+HC+$MgSO_4$ rats compared to RUPP+HC rats (3. 81 ± 1.19 *vs* 1.78 ± 0.65 μg/L, p < 0.05) (Figure 13).

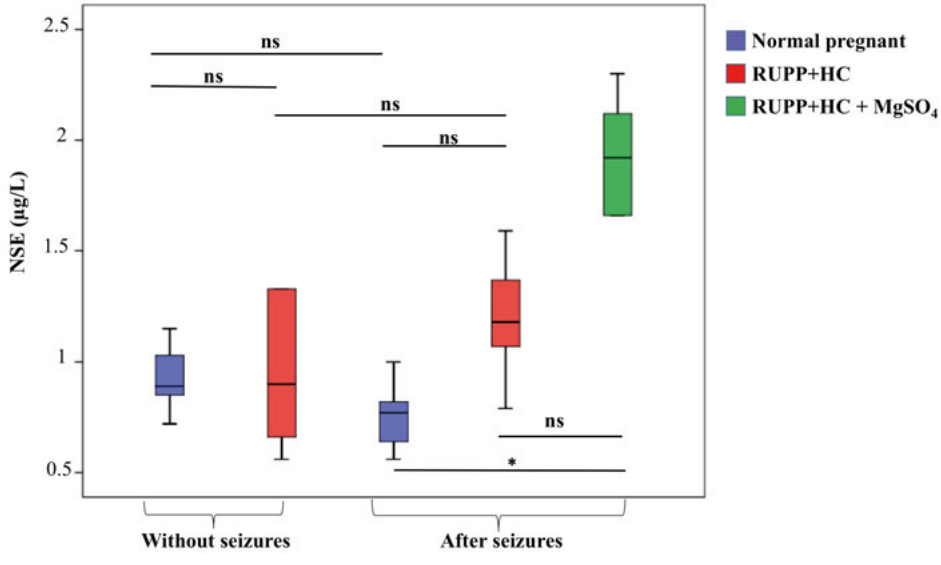

Figure 14. Boxplot of serum concentrations of NSE (μg/L) in normal pregnant rats, RUPP+HC rats and RUPP+HC+$MgSO_4$ rats respectively.

There were no differences in serum concentrations of NSE in normal pregnant compared to RUPP+HC rats (0.90 ± 0.36 vs. 0.89 ± 0.17 μg/L, p = 0.92) (Figure 14). Seizures did not affect circulating NSE during normal pregnancy, as concentrations were similar between pregnant rats with and without seizures (0.77 ± 0.17 vs. 0.89 ± 0.17 μg/L, p = 0.11). Similarly, seizures did not affect circulating NSE in RUPP+HC rats, with RUPP-HC rats having similar serum concentrations of NSE with and without seizures (1.18 ± 0.30 vs. 0.9 ± 0.36 μg/L, p = 0.25) (Figure 14). However, there was an increase in serum concen-

trations of NSE among RUPP+HC+$MgSO_4$ rats and RUPP+HC rats compared to normal pregnant rats post-seizure ($p < 0.05$). After correcting for multiple testing between groups, NSE concentrations remained significantly increased in RUPP+HC+$MgSO_4$ rats compared to normal pregnant rats (1.92 ± 0.57 vs. 0.77 ± 0.17 μg/L, $p < 0.01$) (Figure 14).

There was no correlation between seizure threshold and serum concentrations of S100B and NSE in any of the groups. In RUPP+HC + $MgSO_4$ rats, the correlation coefficient was 0.8 but was non significant ($p = 0.20$).

There was a significant negative correlation between brain water content and concentrations of S100B in RUPP+HC+ $MgSO_4$ rats ($r = -1.0$, $p < 0.01$). In RUPP+HC rats and normal pregnancy, no correlation was found. Regarding NSE, there was no correlation between serum concentrations of NSE and brain water content in any of the groups.

Discussion

In this thesis, we have shown that the cerebral biomarkers S100B and NSE are increased in women with preeclampsia during the disease and one year postpartum and that increased concentrations of S100B correlate to visual symptoms, making S100B and NSE possible predictors for short- and long-term cerebral adverse events in preeclampsia.

There are currently no reliable objective markers to predict the onset of convulsions or other cerebral complications in women with preeclampsia. Neurological symptoms and altered tendon reflexes have poor sensitivity and specificity and are not recommended as the only parameters to act on concerning decisions about treatment, level of care or delivery.[32, 174] Even so, these are the only predictive tools that exist in clinical use for prediction of cerebral adverse events when assessing a woman with preeclampsia or imminent eclampsia and when making the decision to deliver or to treat with $MgSO_4$. In American guidelines, visual or cerebral disturbances are included as one of six criteria to define severe disease.[33, 175]

Should one even screen for eclampsia? According to guidelines for screening (see Introduction, Prediction of eclampsia), eclampsia definitely meets two criteria; it's an important health problem and it has an effective intervention ($MgSO_4$) that results in a better outcome. The problems are that there is no suitable test and we don't know if the benefits of screening outweigh the potential harm regarding maternal and perinatal morbidity since there are no validated screening models. Lastly, there should be a latent or early symptomatic stage which is problematic in eclampsia since today's predictive tools have both poor sensitivity and specificity.

New prediction models including sFlt-1 and PlGF together with clinical signs are emerging to predict adverse maternal and neonatal outcome but cerebral outcomes are rare in these populations. Only a few of these models have been externally validated.[33, 35] In a register-based study from Finland, only 7% of women with eclampsia had received $MgSO_4$ treatment before seizures, a finding that was confirmed in a British register based study where the number was 6% and in this study, 21% of women were at home at the time of their first

fit.[7] This reinforces the difficulty in predicting which women will proceed from preeclampsia to eclampsia.[176]

In preeclampsia it is not yet known whether neuronal damage occurs. Most research implicates that women with preeclampsia and eclampsia suffer from an impaired BBB.[56, 164] The secretion of cerebral biomarkers through the BBB could either be a part of the general endothelial dysfunction and isolated BBB compromise or this in combination with neuronal or glial compromise, alternatively confounded by extra-cerebral sources.[90] Since the present evidence supports that a compromised BBB will eventually lead to neuron and glial injury, the probable scenario in preeclampsia is that a compromised BBB during the course of pregnancy will eventually lead to cell injury. Women with previous preeclampsia and preeclampsia exhibit cognitive impairment and cortical volume loss months to years after pregnancy (see section in the Introduction "Long-term cerebral outcome after preeclampsia and eclampsia"), a finding that supports the theory regarding cellular injury in the CNS in women with preeclampsia.

Methodological considerations

Study populations

Papers I and III

The study design for Papers I and III was a nested case-control study within a cohort study. One disadvantage with the design of this study is the low incidence of preeclampsia, about 4% in this cohort, which requires many subjects enrolled to achieve sufficient power. The other disadvantage is that no woman in this cohort experienced cerebral complications of preeclampsia. Such a study with the same design would require a much larger sample size. Therefore, no conclusions about prediction of neurological complications can be drawn from this population.

The controls selected from the population did not develop gestational hypertension. The weakness of such a design could be that one overestimates the results since, for example, hypertension could be a contributor to increased concentrations of S100B and NSE, and comparing women with hypertension and women with preeclampsia could render another result.

Papers II and IV

A case-control design facilitates more women with preeclampsia to be included, increasing the power of the study. Therefore, we could also study the

relationship between increased concentrations of S100B and visual symptoms as a sign of cerebral involvement. Also in this population, none of the women experienced cerebral complications of preeclampsia. In Paper II, symptoms are collected from medical charts and not retrospectively, limiting the risk of recall bias.

In Paper IV, the same population was also studied one year postpartum as a follow up. The advantage of this is that one can compare the same individual as its own control, eliminating the potential variance between individuals.

Paper V

This study design was an experimental animal study with five animals in each group. The advantage with an experimental design is that one can create similar groups, limiting the degree of confounding. Another advantage is that it is possible to study rare conditions that in a study of humans would require a large sample size for a cohort study or a long inclusion period for a case-control study. A third advantage is that one can study outcome measures that cannot be studied in humans, for example, water content as a proxy for edema and immunohistochemistry on brain tissue for the evaluation of BBB integrity. In Paper V, some of these problems could be addressed and investigated due to the experimental animal design.

The limitations to this design are that it is not always possible to mimic the true state of the disease that one wants to study. Preeclampsia is a disease unique to humans, and even though one can achieve a majority of the phenotype parameters in an animal model, it is still not the true pathophysiology of the disease. Therefore, the results may not be generalizable to the human population.

Sample size

Generally, for all studies, power calculations were not possible since the inclusion of women for Paper I-IV[177, 178] and the experiment for Paper V[45] were already accomplished.

For studies I and III, the initial power calculation was based on the development of preeclampsia and not for cerebral complications and with an incidence of 3–5%, 469 women were recruited to include 20 women with preeclampsia. This study must be considered as a pilot study, since no previous data existed about the plasma concentrations of S100B and NSE in women developing preeclampsia. We could see a difference in plasma concentrations between preeclampsia and healthy pregnancies, allowing us to draw the conclusion that the sample size was large enough for this purpose.

With the results from Papers I and III, we assumed that we would find a significant difference in plasma concentrations of S100B and NSE in Papers II and IV, as this was a larger population. The borderline significant results when adjusting for headache regarding visual disturbances and S100B might be a power-related issue. In another case-control study, 19/26 patients with severe preeclampsia experienced headache or visual symptoms, and in that study, women with neurological symptoms had significant greater odds of having higher plasma S100B concentrations, strengthening our findings.[107] Also, headache might not be a confounder to the presence of visual disturbances but rather a collider, questioning the components in the regression analysis.

For study V, the three Rs—replacement, reduction and refinement had been followed. Five animals per group were chosen. Since there were only five animals in each group, the non-statistically significant difference in serum concentrations of S100B between normal pregnancy and preeclampsia could be power-related, but it would have to be tested in a future study for us to be able to draw any conclusions.

In general, if one wants to find a small difference between two groups with a small alfa (risk of rejecting the null hypothesis despite that it is true) and a large beta (chance of finding a difference when there is a difference), one must increase the sample size. In our material, it is not possible to estimate what difference in the μg/L of S100B and NSE would be of clinical importance, and therefore it is hard to make any power calculations. This difference must be estimated by earlier observations and clinical implications.

Experimental considerations

ELISA

Inter- and intra-assay coefficient of variation

When performing an ELISA, different errors can occur along the way. When comparing different manufacturers, the concentrations obtained from the same sample can vary. The concentrations can also vary between different wells on the same plate (intra-assay variability) or between plates (inter-assay variability) within the same manufacturer. Different batches of ELISA plates from the same manufacturer may also vary between batches. An ELISA set of the same batch with an intra- and inter-coefficient of variation of less than 5% is considered acceptable. However, one should be aware of this variance and take it into consideration when interpreting results. To achieve as reliable results as possible one should use ELISA plates from the same batch and run samples in duplicates or triplicates over different plates. When comparing two groups in a study, the most important component is to use the same batch and

to run cases and controls randomly over the different plates. In our studies, the same manufacturer for S100B and NSE ELISA have been used but different batches for different studies. Therefore, the results are more comparable within studies than between studies.

In Paper I for S100B, the inter- and intra-assay coefficients of variation were, respectively, 4.6 and 3.1%, in Paper II they were 3.3 and 7.7%, in Paper IV they were 3.3 and 7.7% and in Paper V they were less than 12 and less than 10%. For NSE, in Paper III, the inter- and intra-assay coefficients of variation were 2.8 and 4.3%, in Paper IV they were 5 and 2.1% and in Paper V they were less than 12 and less than 10%, respectively. Thus, the coefficients of variation are larger in the animal study, making those results less reliable and less reproducible.

For S100B and NSE, standardized laboratory analyses have emerged and in future analyses, this could be an option to reduce the variance in the results and thus make the analyses more reproducible.

Monoclonal and polyclonal antibodies

Antibodies targeting specific proteins or antigens are produced by lymphocytic B-cells and can be produced either in a living animal, resulting in polyclonal antibodies, or in a cell line, resulting in monoclonal antibodies. Polyclonal antibodies are produced predominately in rabbits after injection of the peptide corresponding to the amino acid sequence of the epitope of interest, and, subsequently, several different B-cells produce antibodies that might have cross-reactivity to other proteins and can thus be less specific. Monoclonal antibodies are derived from one cell line and can be designed to target one or more epitopes on the protein of interest. These are more specific and predictable. Furthermore, monoclonal antibodies are constant and have a low batch-batch variability. Though, polyclonal antibodies can offer a more robust detection since they bind several different epitopes on the surface of the antigen. This is an advantage in immunoprecipitation but can be disadvantageous when measuring concentrations, making them less favourable as capture antibodies.[179]

For the S100B assay in this thesis, two mouse-derived monoclonal antibodies that were directed towards two different epitopes on the S100B monomer were used to coat the wells (capture antibodies) and one monoclonal mouse anti S100B as detection antibody. For the NSE assay, one recombinant monoclonal antibody was used as primary antibody, directed towards one epitope on the γ subunit and the detection antibody added after binding of NSE to the primary antibody was polyclonal.

Both S100B and NSE exist as homo- and heterodimers and the ELISA will detect the monomer of interest both in the case of a homo- and a heterodimer. Therefore, there is a risk that one will pick up a signal from S100B or NSE not deriving from cerebral sources but rather from peripheral sources (see Introduction). For NSE, no commercial ELISA kit exists that only targets the γγ homodimer. For S100B, there are different commercially available ELISA kits. Most detect both S100BB and S100A1B through monoclonal antibodies. S100A1B is found in the heart, striatal muscle and the kidneys, whereas S100BB is predominately found in glial cells and to a lesser extent also in adipocytes, chondrocytes and melanocytes. The ELISA from Fujirebio (Gothenburg) was designed to detect S100A1B and S100BB separately. The method was based on two monoclonal antibodies directed towards S100BB, where one of them showed very little reactivity to S100A1B. The location of the epitope for the second monoclonal antibody was not known, but it could have been that it bound onto both S100B monomers in the dimer. Alternatively, the antibody could have had a structurally dependent epitope that was only present in the S100BB dimeric form (personal communication with Christian Fermér, Research and Development Director, Fujirebio). This has, thus far, shown no advantage over analyzing S100B regarding outcome after severe traumatic brain injury.[114, 180] The company no longer provides this targeted S100BB assay—only a monoclonal assay detecting S100BB and S100A1B together, similar to the ELISA used in this thesis (personal communication with Christian Fermér, Research and Development Director, Fujirebio).

Knowing that S100B is also produced in adipose tissue, we adjusted for BMI, age and parity in Paper II, and S100B remained an independent risk factor for preeclampsia. However, one must keep in mind that preeclampsia is a generalized endothelial disease and that the risk of confounding sources might be larger than in other diseases with cerebral pathology. Therefore, if we were to have had access to an ELISA directed to the NSE γγ homodimer or S100BB homodimer, it could potentially have diminished the contribution and confounding from extra-cerebral sources to the plasma concentrations.

For NSE, the manufacturer recommends Li/Hep tubes for plasma collection and recommends an additional centrifugation to remove all trace of platelets, since NSE is also found in platelets and, if not removed, will confound the results. We used Li/Hep tubes, but the samples were only centrifuged once. This might lead to a remaining trace of platelets in the samples, possibly overestimating the NSE concentration in our samples. This finding, though, would be similar between groups or increased in healthy controls, since preeclampsia is known to have a lower rather than a higher platelet count. In Paper III, we

also adjusted for LD as a marker for hemolysis, although no clinical or visual hemolysis was present.

The ELISA assays used both for S100B and NSE are commercial ELISAs that have previously been used in similar analyses, and the ELISA assay for S100B is the same as that used in two of the three previous studies evaluating S100B in preeclampsia, making the results comparable.[107-109]

Time to analysis

The ELISA analyses were done several years after the sampling. The plasma samples were frozen directly after venipuncture and only thawed for the respective ELISA analyses. The majority of proteins are known to be stable in the frozen condition (communication with the chemical laboratory at Uppsala University Hospital), and since the samples were handled in a standardized manner the error should be similar between cases and controls.

Statistics

For Paper I, Bonferroni correction was not used for the dependent sample testing, increasing the risk for type 1 error. However, when the sample size is small, the risk of using Bonferroni correction is to obtain a type 2 error, since it is a fairly blunt test. In Paper II the result of increased concentrations of S100B in preeclampsia is confirmed, strengthening the results from Paper I. In Paper III, Bonferroni correction was used with where statistically significant differences between time points remained in gestational week 37. If Bonferroni correction would not have been used, there would also have been a statistically significant difference between groups in gestational week 33. For the population in Papers I and III, there were relatively few cases, but despite that we reached significant results in our outcomes, indicating a strong association.

For all statistical analyses, there is always the risk of a type 1 or a type 2 error, since the p-value is chosen at 0.05. Choosing this p-value will, statistically, mean that the results from 1 out of 20 studies will not be the true result. The fact that all the studies in this thesis support the assumptions that concentrations of S100B and NSE are increased in women with preeclampsia supports these assumptions as being correct.

Confounders for Paper II, III and IV were chosen based on the underlying confounding sources of S100B and NSE. In Paper II and IV, adjustments were also made for common background characteristics such as age.

Cerebral biomarkers

S100B is an astrocyte-derived protein and is released from astrocytic end-feet.[181] As presented in the Introduction, the astrocytic end-feet make up a part of the NVU, meaning that the production and release of S100B are close to the BBB and thus makes S100B a plausible biomarker for BBB impairment. The initial increase in S100B could reflect the BBB compromise; but in a later stage, increased concentration of S100B could also reflect a glial injury secondary to the BBB impairment and edema formation.[90]

NSE is a neuronal protein, and it has been proposed that increased plasma concentrations of NSE are due to neuronal injury rather than isolated BBB compromise.[112, 113] Regarding the fact that women with preeclampsia might suffer from BBB injury, one can hypothesize that NSE is released into peripheral blood after a period of time after initial BBB injury, according to Figure 5B in the Introduction. In Paper III, plasma concentrations of NSE differed significantly between groups only in gestational week 37, which is one month later than when levels of S100B differed between women developing preeclampsia and women with normal pregnancies in Paper I, supporting this theory.

To answer the question of whether S100B and NSE have the characteristics of ideal cerebral biomarkers in preeclampsia, one can refer to the characteristics discussed in the Introduction. NSE and S100B represent glial and neuronal cells and are therefore representative for the pathophysiology behind the condition. They are not specific to the disorder, since S100B and NSE are also found in extra-cerebral sources. The important question is whether or not this contribution is of importance, and to answer that question one must examine a population with adverse cerebral outcome. In Papers I and III we showed that S100B and also probably NSE appear before the clinical onset of disease. For preeclampsia, both S100B and NSE display fairly good discrimination between cases and controls, but here the most interesting question to answer is whether they discriminate as well between women with preeclampsia and adverse cerebral outcome compared to women with preeclampsia with a low risk for adverse cerebral outcome. Concentrations of S100B have shown to correlate to severity of the disease in one study,[109] and furthermore, in Paper II and in an additional case-control study, plasma concentrations of S100B correlate to the presence of cerebral symptoms.[107] In normal conditions, both S100B and NSE are detected in low concentrations in peripheral blood.[110]

In summary, S100B and NSE do not fulfill all criteria for ideal biomarkers in preeclampsia and cerebral complications but show promising features and only future studies with endpoints such as eclampsia, PRES or intracerebral

bleeding as short term outcome or cognitive disorders or dementia as longterm outcome can answer that question.

Alternative sources of S100B and NSE

Plasma volume

In normal pregnancy, systemic vascular dilatation leads to increased intravascular volume. The plasma component contributes most to this increase in volume.[182] Hemoglobin content increases by 18–27%, resulting in a relative dilution, and the lower limit of normal Hb in pregnancy is 11 g/dl.[183] In a meta-analysis, plasma volume increased by 0.18 L (7.7%) in the first trimester, by 0.95 L (40%) in gestational weeks 22–28, by 1.09 L (43.1%) in gestational weeks 29–35 weeks and by 1.15 L (45%) in gestational weeks 36–41[184] (Figure 17).

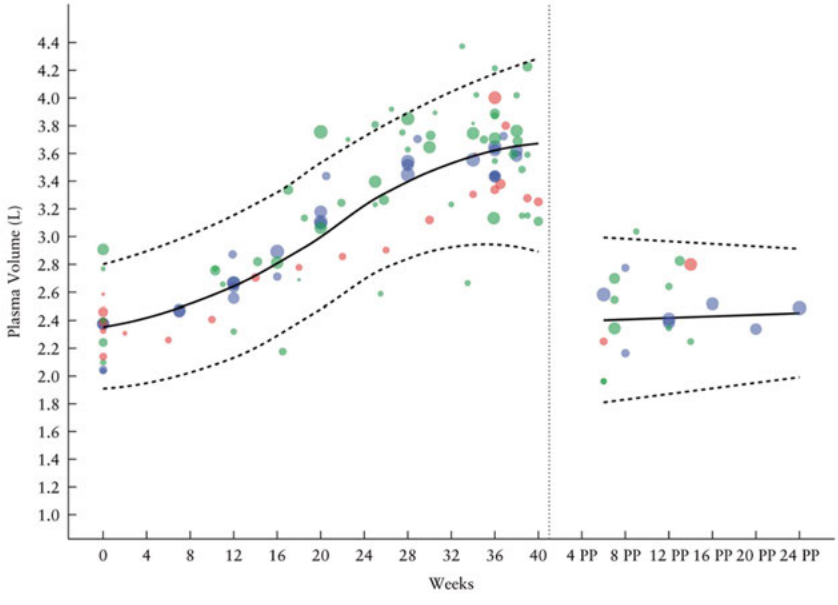

Figure 17. Plasma volume expansion in normal pregnancy. Reproduced from Ultrasound in Obstetrics and Gynecology with permission from Wiley.

Fewer studies examined the plasma volume expansion in women with preeclampsia, and the results should be interpreted with caution. One study showed a plasma expansion of 0.53 L (25%) at gestational week 26. In gestational weeks 32–34, the increase was 0.93 L (32%). In gestational weeks 36–41, more studies were eligible, and a pooled increase of 0.8 L (33.3%) was found, which was significantly less, by 13.3%, than in healthy pregnancies ($p < 0.0001$).[184]

Knowing that women with preeclampsia experience lower plasma volume compared to women with healthy pregnancies, the concentration of cerebral biomarkers might be increased due to the lower plasma volume in women with preeclampsia. In Papers I and III, we adjusted for Hb as a measurement of hemoconcentration in gestational week 37, and plasma concentrations of S100B and NSE remained significantly increased among women with preeclampsia in contrast to controls (data not shown). In Papers II and IV, correlation between EVF as a proxy for hemoconcentration and concentrations of S100B and NSE in women with preeclampsia did not show any correlation (data not shown).

Our results further show that the difference in plasma concentrations of the biomarkers occurs between gestational weeks 33–37. According to the meta-analysis, the greatest changes in plasma volume occur until gestational week 28,[184] and therefore, one should be able to detect a difference in the plasma levels of the cerebral biomarkers by that time if the studies demonstrating lower plasma expansion in women developing preeclampsia by that time reflect the true values. In contrast, in both Papers I and III, the plasma concentrations of S100B and NSE were similar between groups in gestational weeks 10, 25 and 28. Further, plasma concentrations between gestational weeks 25 and 28 were at levels similar to those in gestational week 10 in both groups, making the relative increase of the amount of S100B and NSE equal between groups.

Extra-cerebral sources

Since preeclampsia is a multifactorial disease that involves many organs, the discussion around extra-cerebral sources is important. We cannot exclude that the increased concentrations of S100B and NSE in women with preeclampsia in our material are due to extra-cerebral sources rather than secretion from the CNS. Our studies have to be regarded as pilot studies for cerebral biomarkers in preeclampsia, and future studies investigating the role of cerebral biomarkers should investigate the predictive ability of the biomarkers for adverse outcome, both in the short- and long-term. If they turn out to be clinically relevant predictors, then the contribution from extra-cerebral sources is of less importance.

S100B

There is an ongoing discussion concerning the contribution of extra-cerebral sources regarding increased plasma concentrations of S100B. This issue has been discussed in the literature in relation to other diseases,[181, 185] but there is evidence that S100B is mainly expressed in astrocytes and that its low expression in other tissues is of less importance clinically when evaluating brain damage.[186] Pham and collegues[187] have in a recent publication shown that a

change in serum concentrations of S100B primarily reflects extravasation across a disrupted BBB.

Increased concentrations of S100B have been demonstrated in the plasma of growth-restricted fetuses,[141] and in pregnancies with chronic fetal hypoxia, increased concentrations of S100B have been found in the amniotic fluid.[188] Increased maternal plasma concentrations of S100B have been found in pregnancies complicated by growth-restricted fetuses and intraventricular hemorrhage in the child.[141] This might be a confounding source to increased plasma concentrations of S100B regarding our studies in pregnant women. There is no evidence that S100B passes the placental barrier, and, therefore, no conclusions can be drawn if S100B concentrations in the feto-maternal unit influence the maternal plasma concentrations. In Paper I, there were no infants born in the study population that were SGA. Therefore, it is not likely that the increased concentrations of S100B in the end of pregnancy among women developing preeclampsia in Paper I are due to contribution from the feto-maternal unit. In Paper III, 10 women in the preeclampsia group gave birth to SGA infants. When excluding these from the analyses, concentrations of S100B were still increased among women with preeclampsia compared to controls (data not shown).

In Paper II, an association with visual disturbances and increased concentrations of S100B was found. The predilection of pathological changes in PRES for the parieto-occipital region, including the visual cortex, corresponds well to the fact that visual disturbances are one of the most common symptoms in PRES.[189-192] There is one previous study supporting the correlation between increased plasma concentrations of S100B and visual symptoms but another study where this association could not be found.[107, 109]

NSE

NSE exists as a homodimer and a heterodimer. The heterodimer exists in the CNS but is also found in DNES cells. DNES cells have been found in the corpus luteum of pregnant dairy goats, and since NSE in goats has a high nucleotide and amino acid sequence homology with NSE in humans, NSE is likely to also be found in the corpus luteum of humans.[193] In humans, the corpus luteum is active during the first 12 weeks of pregnancy, and this could at least partially explain the somewhat increased concentrations of NSE in early pregnancy that was found in the population in Paper III. It might also explain why plasma concentrations of NSE decrease throughout pregnancy in healthy controls when the corpus luteum is no longer actively secreting NSE. If so, the persistent increased concentrations of NSE in women with preeclampsia could be explained by the secretion of NSE through an altered BBB.

A number of studies have demonstrated increased concentrations of NSE in plasma in the case of hemolysis, as NSE also can be found in red blood cells.[145] Pelinka et al. demonstrated a two- to three-fold increase in serum-NSE concentrations secondary to isolated ischemia and reperfusion in abdominal organs in rats and also an increase in serum-NSE concentrations among trauma patients without traumatic brain injury.[143] When adjusting the results in Paper III for hemolysis by using concentrations of lactate dehydrogenase as a marker for hemolysis, the concentrations of NSE in gestational week 37 in women who developed preeclampsia were still increased over healthy pregnant controls. This would support that the difference in NSE concentrations between women with preeclampsia and women with healthy pregnancies is not due to hemolysis, a condition that is more common among women with preeclampsia.

Concentrations of NSE in umbilical cord blood is about three times as high as in maternal blood in healthy pregnancies from our cohort study (Paper III), but this comparison should be interpreted cautiously since ELISA kits have inter-assay variations, and populations should only be compared using the same assay and the same batch.

$MgSO_4$

In Paper V, concentrations of both S100B and NSE were increased in experimental preeclampsia with $MgSO_4$ treatment post-seizure compared to experimental preeclampsia without treatment and normal pregnancy respectively. These results are in conflict with previously published results showing a protective function of $MgSO_4$ on the BBB.

Animal models of traumatic brain injury, sepsis and hypoglycemia show increased edema and altered BBB, an effect that is reduced with $MgSO_4$ treatment.[102, 194-196 197] In the rat model of severe preeclampsia used in Paper V, $MgSO_4$ reduced the seizure threshold and reduced neuroinflammation. The brain water content was less in preeclampsia compared to healthy pregnancy and was not affected by $MgSO_4$ treatment. In preeclampsia, there was an increased concentration of the 470 Da sodium fluorescein stain in preeclampsia, unaffected by $MgSO_4$ treatment, and no difference could be seen between healthy pregnancy, preeclampsia and preeclampsia with $MgSO_4$ treatment regarding permeability for the larger 70 kDa dextran.[45] These findings implicate that the BBB protective action seen in other brain injury models might not be the mechanism of action regarding the effect of $MgS0_4$ as seizure prophylaxis in eclampsia.

The actions of $MgSO_4$ to reduce seizure activity in eclampsia are not known. The protection of the BBB seen in other animal models for brain injury has not, thus far, been reproduced in preeclampsia models. The higher peripheral concentrations of S100B and NSE in MgSO4 treated preeclampsia rats could be due to 1) an increased secretion of S100B and NSE through a more compromised BBB in $MgSO_4$ treated rats, 2) an increased neuronal and glial injury in $MgSO_4$ treated rats or 3) increased secretion of S100B and NSE from peripheral sources in $MgSO_4$ treated rats. The findings of increased peripheral concentrations of cerebral biomarkers in MgSO4 treatment in Paper V warrant further investigation into the role of MgSO4 in seizure prophylaxis in preeclampsia.

Intra-cerebral Mg^{2+} levels have been shown to be reduced in women with preeclampsia in contrast to healthy pregnant controls.[47, 198] In rat models of brain injury, increased intra-cerebral Mg^{2+} levels are observed after treatment with $MgSO_4$.[195] In a study of PRES including different etiologies, the authors found hypomagnesaemia in all 19 patients examined, and the hypomagnesaemia started at the same time as the onset of PRES.[199] Together these findings implicate that depleted intra-cerebral Mg^{2+} levels in women with preeclampsia might be one of the reasons as to why treatment with $MgSO_4$ is efficient in reducing eclampsia by the restoration of intra-cerebral $MgSO_4$ levels and possibly antagonistic action on the NMDA receptor.

In conclusion, the ability of $MgSO_4$ to reduce seizures in severe preeclampsia and eclampsia seems to work through a different pathway than BBB protection. The reduction of neuro-inflammation and the restoration of intra-cerebral Mg^{2+} levels are two different hypotheses. Knowing that NNT for preventing one case of eclampsia in women with severe preeclampsia is 71,[17] $MgSO_4$ treatment is probably efficient only if the cause behind the convulsions is due to one of the above reasons. Though, the finding of increased serum concentrations of both S100B and NSE in $MgSO_4$ treated animals warrants further investigation as to the intra-cerebral action of $MgSO_4$ in preeclampsia and eclampsia. Therefore, further research is needed to elucidate the role of $MgSO_4$ in the prevention and treatment of eclampsia.

The animal model

Animal models of preeclampsia have been criticized due to the human-specific nature of preeclampsia. Different animal models cover different aspects of preeclampsia, ranging from placental defects to maternal end organ injury, and it is important to choose the correct model when conducting preeclampsia research. In this thesis, we have been concentrating on cerebral involvement

in preeclampsia, and we have used models visualizing the cerebral complications in preeclampsia. The RUPP+HC model was specifically developed to visualize the endothelial injury, including BBB disruption, in preeclampsia[45] and should therefore be suitable for the investigation of cerebral biomarkers.

In the animal model, the concentrations of S100B did not differ between normal pregnancy and preeclampsia, though there was a tendency toward increased concentrations of S100B in rats with induced preeclampsia. This can be due to the low number of subjects thus not reaching enough statistical power or due to the difference between the human disease and the animal model. Normal pregnant animals with induced seizures had increased concentrations of S100B compared to normal pregnancy without seizures, supporting a seizure-induced BBB compromise in pregnancy. This was not found in preeclampsia, which implicates that concentrations of S100B are not further increased after seizures. The reason for this cannot be determined in this study, but one can speculate about whether the secretion of S100B through an already compromised BBB reaches a steady state before seizures occur.[90] An alternative explanation could be that the model where seizures are induced by PTZ does not completely reflect the preeclampsia–eclampsia state and that the PTZ model might not reflect the ongoing disruption of the BBB and successive increase in intra-cerebral vasogenic edema seen in eclampsia.[60] A third explanation could be that S100B concentrations are different between individuals with preeclampsia. In our studies, a greater variance in the levels of S100B is seen in women with preeclampsia compared to healthy controls, and therefore it might be that one needs to examine the S100B concentrations before, during and after seizures in the same individual to be able to draw any conclusions.

In the animal model, NSE did not differ between groups, neither between normal pregnancy and preeclampsia nor between animals with and without seizures. These findings are supported by BBB studies that show that NSE is not increased in peripheral blood in isolated BBB compromise.[90] However, the results are conflicting with the findings in Papers III and IV, where plasma concentrations of NSE were increased in women with preeclampsia. This could be due to the animal model where either the secretion from peripheral sources is less or the neuronal injury is less pronounced. The lack of increase in NSE concentrations in animals with induced seizures could implicate that a potential isolated BBB injury and seizures in the animal model do not include neuronal injury.

We found no correlation between seizure threshold and serum concentrations of NSE and S100B, though there was a tendency toward a correlation between increased serum levels of S100B and lower seizure threshold in

RUPP+HC+$MgSO_4$ rats. The correlation was strong, with an r^2 of 0.82, indicating that a large proportion of the seizure threshold could be explained by serum S100, but it might be of interest to look further into this relationship with a larger number of subjects.

For brain water content, there was a significant correlation between increased serum concentrations of S100B and lower percentage of water content in the brain. This is interesting, since one would expect the opposite—that a more severe damaged BBB would let more S100B out into the circulation and more water into the brain. Though, when comparing rats with experimental preeclampsia to rats with normal pregnancies, the rats with experimental eclampsia had a lower brain water content compared to normal pregnant rats with induced seizures.[45] Thus, the pathophysiology behind eclampsia might be more complicated than previously thought, and the lower plasma volume and distorted fluid balance in preeclampsia and eclampsia might play a role.[184]

The finding that these two correlations were seen only in $MgSO_4$ treated rats is surprising and in agreement with the increased serum concentrations of both S100B and NSE in MgSO4 treated rats. This would support the idea of an unknown intra-cerebral mechanism of $MgSO_4$ that requires further investigation.

Implications

Do cerebral biomarkers reflect cerebral involvement in preeclampsia?

The increased plasma concentrations of both S100B and NSE weeks before median time of onset of clinical signs and symptoms in women developing preeclampsia implicate that there is a neurological compromise early in the course of the disease. The cerebral function before onset of preeclampsia is poorly investigated but the knowledge of alterations in other organ systems such as the cardiovascular system with increased vascular resistance, decreased cardiac output and impaired relaxation[19] supports the idea that cerebral function also might be altered before diagnosis. Furthermore, pregnancy itself seems to remodel the cerebral vasculature, potentially making it more vulnerable to endothelial injury, also supporting our findings.[55]

In Paper IV, we showed that both NSE and S100B concentrations are increased in women with previous preeclampsia also one year after delivery. In this population, the NSE and S100B concentrations were not known before pregnancy, but in the population of Papers I and III the concentrations in early pregnancy did not appear to differ between groups. These populations are fairly similar, and one can assume that the population in Paper IV would also not differ in concentrations of S100B and NSE during early pregnancy. If this is true, there might be persistent brain involvement in women with previous preeclampsia years after pregnancy. As discussed in the Introduction, there is some evidence of cerebral lesions and cognitive function impairment in women with preeclampsia months to years after pregnancy and that lesions correlate with time from pregnancy.[79, 81-83] This is in agreement with our findings that suggest BBB compromise and/or intra-cerebral involvement also postpartum in women with previous preeclampsia. White matter lesions occur predominately in the frontal lobes, whereas PRES is mainly found in the parieto-occipital lobes, suggesting that there are different pathophysiological pathways.[60, 68-71, 79] However, this is mainly a speculation, since no longitudinal studies in women with preeclampsia and PRES exist that compare PRES and severity of edema to long-term neurological outcome and white matter lesions. In addition, recent studies have proposed that PRES is found also in

the frontal lobes in a large proportion of women with preeclampsia or eclampsia, strengthening the correlation between PRES during pregnancy and white matter lesions later in life.[69, 72]

Can cerebral biomarkers predict eclampsia in women with preeclampsia?

This thesis does not include human populations with cerebral complications of preeclampsia. Therefore, the ability of S100B and NSE to predict eclampsia or other adverse cerebral events in women with preeclampsia cannot be determined within the scope of this work. The animal study implicates that S100B might predict seizures in normal pregnancy, but this could not be seen in preeclampsia.

Current evidence does not include prediction models of eclampsia. The PIERS, PETRA and PREP studies together with studies of prediction with biomarkers yield AUC:s of 0.8-0.85 for adverse maternal outcome but adverse cerebral outcomes are rare and the most common adverse outcome is delivery before 34 weeks and therefore, the predictive value for eclampsia, PRES and cerebral bleeding is uncertain.[35] There are also uncertainties around the clinical implications with these screening models where a concern is that neonatal morbidity and mortality due to iatrogenic preterm delivery might be a consequence and no model has been evaluated in clinical practice. An ongoing randomized study in Ireland, the PARROT study, will include women with suspected preeclampsia and treat them according to routine care or according to PlGF concentrations and subsequently evaluate maternal and neonatal morbidity and also health economic impact. This will give more information about the usefulness of existing prediction models but probably not regarding the prediction of cerebral adverse outcome and therefore will not be able to aid in the reduction of NNT for treatment with $MgSO_4$ for avoiding eclampsia.

An ideal study design would prospectively follow women with preeclampsia with serial blood samples until discharge from the hospital. For the women developing eclampsia, one could follow the dynamics of the cerebral biomarkers and evaluate their ability to predict adverse cerebral events. Unfortunately, this design is hard to achieve, since many women with eclampsia present with eclampsia upon admission, and women who are admitted and offered expectant management are often delivered before the onset of cerebral complications. A retrospective cohort study from Egypt showed that delay to presentation and undiagnosed preeclampsia without proper antenatal care are the main risk factors for mortality in women with hypertensive disorders.[12] In

Sweden and other European countries, the incidence of eclampsia is low compared to developing countries.[8, 11, 14] To set up a cohort for the prediction of eclampsia in Sweden would require the inclusion of thousands of women. In certain developing countries, the eclampsia rate is higher, but the antenatal programs are not as well established, which makes it challenging to find the women at risk.

Cerebral biomarkers as long-term predictors in women with previous preeclampsia

There are no present predictors for long-term neurological outcome in women with preeclampsia. It is unknown whether PRES is a completely reversible condition in women with preeclampsia. No studies have explored the association between PRES and long-term neurological outcome. It would be of interest to correlate the peripheral concentration of cerebral biomarkers to the presence of PRES, cognitive scoring, cerebrovascular events and dementia later in life. It would also be of interest to evaluate the true incidence of PRES among eclampsia and preeclampsia with neurological symptoms. At present, there are mainly retrospective studies of preeclampsia and eclampsia and PRES where imaging has been indicated but few prospective studies and no studies correlating PRES to cerebral outcome. One problem with cerebral biomarkers as predictors for long-term cerebral outcome would be the lack of a well defined treatment in an early stage that would provide a better outcome for the women at risk.

This thesis does not include papers that answer the question as to whether cerebral biomarkers during pregnancy can predict the occurrence of intra-cerebral lesions or cognitive function from the long-term perspective. Such a study would have to be a follow-up study from populations either from Papers I and III or II and IV with cerebral imaging or alternatively a new cohort study of women with preeclampsia or eclampsia. Also, in Paper IV we could not exclude the possibility that the concentrations of S100B and NSE were due to peripheral contributions from extra-cerebral organs.

Conclusions

Paper I

S100B plasma concentrations increase among women who develop preeclampsia compared to women with normal healthy pregnancies, and the increased concentrations precede the onset of disease. S100B is a potential peripheral biomarker reflecting cerebral involvement in preeclampsia.

Paper II

S100B is increased among women with preeclampsia compared to healthy pregnant controls. Increased concentrations of S100B among women with preeclampsia associate with visual disturbances, which might reflect possible CNS effects.

Paper III

Concentrations of NSE are increased in late pregnancy among women who develop preeclampsia compared to women with normal healthy pregnancies. In women developing preeclampsia, concentrations of NSE do not change during pregnancy in contrast to women with healthy pregnancies, where concentrations of NSE decrease throughout pregnancy. This result might be confounded by extra-cerebral sources in early pregnancy, such as the corpus luteum.

Paper IV

Concentrations of NSE and S100B remain increased one year after delivery in women with previous preeclampsia in contrast to women with previous healthy pregnancies. The difference is significant also after adjustments for the confounders age, BMI, parity and days after delivery. The biomarkers correlate in women with preeclampsia but not in normal pregnancies. The sources of the increased concentrations in preeclampsia cannot be determined in this study.

Paper V

S100B but not NSE was increased in an experimental model of preeclampsia in pregnant animals only after seizure, suggesting it may be a useful biomarker for brain injury in pregnancy. Cerebral biomarkers did not correlate to brain water content in the posterior cortex or seizure threshold, although it should be noted that the sample size was low. Treatment with $MgSO_4$ was associated with increased concentrations of both S100B and NSE, a finding that warrants further research as to the safety of the use of $MgSO_4$ as a treatment for imminent eclampsia.

Future research

MRS and cerebral biomarkers

We have conducted a cross-sectional MRS study on women with preeclampsia (n = 30), women with normal pregnancies (n = 30) and non-pregnant women (n = 11) where all women answered a questionnaire about cerebral symptoms, underwent a brain MRI and MRS examination and left blood samples for later analyses.

With MRS, it is possible to measure the levels of different metabolites in a volume. The most commonly studied atoms are hydrogen (H-MRS) and phosphorus (P-MRS). To evaluate the energy metabolism, P-MRS is of special interest, but this technique is not as widespread as H-MRS. P-MRS reflects the cell's energy metabolism. MRS, in contrast to MRI, can detect biochemical dysfunction before morphological changes appear that are visible with MRI.[200]

The primary findings of this study concerning magnesium levels in the brain are discussed in the Introduction.[47] The blood samples of the women were analyzed for concentrations of S100B and NSE with the same ELISA kits as in previous studies.

Preliminary results show that both plasma concentrations of S100B and NSE were increased in women with preeclampsia compared to women with normal pregnancies ($p < 0.01$), confirming the findings in earlier studies. Increased concentrations of NSE correlate to higher intra-cerebral concentrations of glutamate in women with preeclampsia ($p < 0.01$). Glutamate is an excitatory substance, and that women with increased concentrations of glutamate demonstrate increased plasma concentrations of NSE is an interesting finding, possibly suggesting that NSE is produced in excessive amounts in neurons of higher excitability and, alternatively, that already affected neurons release more NSE into the circulation and might suggest subtle neuronal injury in women with preeclampsia.

In vitro models and cerebral biomarkers

In collaboration with MD PhD Carlos Alonso at the University of Bio-Bio, Chillan, Chile, a model of BBB integrity in preeclampsia is being developed with *in vitro* experiments. Plasma samples from women with preeclampsia, healthy pregnancies and non-pregnant women from the MRS study will be added to human brain endothelial cells *in vitro,* and permeability and VEGF expression will be investigated. The results will be compared to concentrations of S100B and NSE in the samples. We will also investigate the direct effect of S100B and NSE on the endothelium by removing the proteins from the samples and rerunning the same analysis. This will provide a better understanding of how the plasma from women with preeclampsia affects the BBB and whether S100B and NSE are a direct cause of the endothelial disruption or whether they are a secondary effect of the altered BBB permeability.

Cerebral biomarkers in women diagnosed with eclampsia

In collaboration with Dr. Catherine Cluver at Tygerberg University Hospital, Stellenbosch University, South Africa and professor Stephen Tong, Melbourne University, Australia, a study protocol for a preeclampsia and eclampsia biobank with ethics approval from Stellenbosch University has been created with Dr. Bergman, Dr. Cluver and Dr. Tong as investigators. A cohort of women with eclampsia and preeclampsia with cerebral complications will be recruited at Tygerberg Hospital. Women will be enrolled in the study at admission to the hospital and followed with serial blood samples throughout their hospital stay. A cross-sectional control group will be recruited simultaneously and consists of women planned for elective caesarian section due to breech or two previous caesarian sections with normal pregnancies.

In this study we will be able to investigate whether cerebral biomarkers are increased in women with eclampsia or preeclampsia with cerebral complications and study the kinetics of the biomarkers throughout the hospital stay. We will also be able to investigate whether increased concentrations of cerebral biomarkers can predict adverse outcomes including PRES, cerebral hemorrhage, GCS score, length of hospital stay, renal failure, pulmonary edema, HELLP syndrome and liver hematoma. Additionally, we will be able to adjust for concentrations of cerebral biomarkers in umbilical cord blood as a confounding source. We will also correlate maternal serum concentrations of cerebral biomarkers and albumin to CSF concentrations of cerebral biomarkers and albumin in women with eclampsia and healthy controls to evaluate the BBB integrity and the roles of the cerebral biomarkers for BBB compromise.

Finally, we will investigate the relationship between serum concentrations of cerebral biomarkers and cognitive function, testing at the event and at follow up after discharge.

Cerebral biomarkers in women developing eclampsia

At Tygerberg Hospital, there are two ongoing RCTs where women with early onset preeclampsia are recruited to evaluate esomeprazole and metformin, respectively, as treatment for preeclampsia. A total of 250 women will be recruited in total. The women are monitored in the obstetric unit at Tygerberg until they reach 34 weeks of gestation or alternatively develop severe preeclampsia, and at that time point they are delivered. Blood samples are taken two times per week, and information about complications is collected prospectively. 120 women are included and three have thus far developed eclampsia. We plan to include the women that develop eclampsia and randomly select triple amounts of control subjects as a nested case-control study for analyzing cerebral biomarkers and their potential in predicting eclampsia in this cohort of women.

Neuron filament light chain (NfL) and tau as biomarkers in preeclampsia

Two biomarkers that derive from neuronal axons, tau and NfL, have been extensively studied in neurodegenerative disorders and in particular in Alzheimer's disease. ELISA assays on the market have not been sensitive enough to detect small concentrations in peripheral blood but recently, newer, more sensitive assays have shown promising results in traumatic brain injury and differentiation for Alzheimer's disease compared to other conditions with similar symptomatology. These assays have now been used to analyze plasma from the same population as in Papers I and III.

Preliminary data show that the concentrations of these biomarkers are increased at the end of pregnancy among women developing preeclampsia. In addition, tau and NfL in combination with S100B and NSE as a predictive model can, in this population, predict the onset of disease in gestational week 25. This implicates that BBB in women with preeclampsia might be affected even before the onset of hypertension and proteinuria.

NfL and tau will be further analyzed in the cohort of women with eclampsia recruited at Stellenbosch University, Tygerberg Hospital in Cape Town, South Africa.

Sammanfattning på svenska

Havandeskapsförgiftning (preeklampsi) är en graviditetsspecifik sjukdom som uppkommer efter den 20:e graviditetsveckan och som drabbar 3-5% av alla gravida kvinnor. Orsaken är inte känd men en huvudhypotes är att det har att göra med en undermålig infästning av moderkakan (placentation) ensamt eller i kombination med vissa riskfaktorer hos modern. Preeklampsi kan involvera många organ i kroppen genom påverkan på kroppens endotel som täcker insidan av blodkärlen.

Diagnosen sätts ofta utan att kvinnan har några symtom och innefattar högt blodtryck i kombination med äggviteutsöndring (proteiner) i urinen. Hos de flesta kvinnor märks sjukdomen endast som högt blodtryck och protein i urinen men vissa kvinnor drabbas av komplikationer till sjukdomen i form av njursvikt, lungödem, lever- och hematologisk påverkan (HELLP syndrom), kramper (eklampsi) eller hjärnblödning. Hos dessa kvinnor är sjukligheten och dödligheten hög, särskilt i utvecklingsländer. Fostret har också en högre risk för påverkan vid preeklampsi i form av tillväxthämning och risk för avlossning av moderkakan. Den enda kända boten mot preeklampsi är förlossning. Högt blodtryck kan behandlas med blodtrycksmedicin för att undvika hjärnblödning och magnesiumsulfat kan användas för att undvika att kvinnan utvecklar eklampsi.

Det finns inga tillförlitliga sätt att avgöra vilka kvinnor som kommer att utveckla komplikationer till preeklampsi. För att ta ställning till vilka kvinnor som behöver behandling med magnesiumsulfat eller botas genom förlossning använder man sig av en kombination av parametrar som innefattar blodtryck, andningsfrekvens, blodets syremättnad, symtom, fosterpåverkan samt vissa blodprover. Problemet är att det är svårt att förutsäga när kvinnan ska råka ut för komplikationer. Prediktionsmodeller för komplikationer utvecklas men har inte inkluderat kvinnor med cerebrala komplikationer. För att undvika eventuella komplikationer beslutar man ibland om förlossning även om barnet är prematurt. Dessutom innebär dagens riktlinjer att vi behöver behandla 71 kvinnor med svår preeklampsi med magnesiumsulfat för att undvika att en kvinna drabbas av eklampsi.

Hjärnan påverkas av graviditet och förlossning och i ännu högre grad hos kvinnor med preeklampsi. Ett skadat endotel innebär att alla kärl i kroppen läcker vätska vid preeklampsi. I hjärnan finns det normalt en barriär, blod-hjärnbarriären, som skyddar hjärnan från substanser i blodet som inte skall komma in i hjärnvävnad. Vid en intakt blod-hjärnbarriär håller det negativt laddade basalmembranet i blod-hjärnbarriären större delen av vätskan i blodbanan genom att hålla kvar ämnen som drar med sig vatten i blodbanan. Hos kvinnor med preeklampsi visar studier att denna barriär troligen är skadad och vätska och andra ämnen kan då komma in i hjärnan. Motsatt kan även ämnen som normalt är hjärnspecifika läcka ut i blodet.

Långtidsprognosen för kvinnor med tidigare preeklampsi innefattar högre risk för hjärt-kärlsjukdom, även innefattande stroke. Det finns studier som visar påverkan på hjärnbarkens volym, ärrbildning i hjärnan och viss kognitiv påverkan hos kvinnor med tidigare preeklampsi när de undersökts månader till år efter graviditeten. Det finns idag inga säkra metoder för att förutsäga vilka kvinnor med preeklampsi som kommer att drabbas av kognitiv påverkan eller annan hjärnpåverkan senare i livet.

Denna avhandling har haft som mål att undersöka om de hjärnspecifika proteinerna S100B och neuron specific enolase (NSE) uttrycks i ökade koncentrationer i blodet hos kvinnor som utvecklar preeklampsi, under och efter graviditeten. Vi har även undersökt hur dessa proteiner beter sig i blodet i en djurmodell av preeklampsi med framkallade kramper samt vid behandling med magnesiumsulfat.

I Studie I och III analyserades blodprover från en studie där man inkluderat 469 kvinnor vid graviditetens start. 20 av dessa utvecklade preeklampsi varav 16 kvinnor hade blodprover tillgängliga för analys. 36 kontroller plockades slumpmässigt ut för jämförelse. Blodprover var tagna i graviditetsvecka 10, 25, 28, 33 och 37 och koncentrationer av S100B och NSE var förhöjda hos kvinnor som utvecklade preeklampsi i graviditetsvecka 33 och 37 för S100B och i graviditetsvecka 37 för NSE, jämfört med friska gravida kontroller. Mediantidpunkt för diagnos var i graviditetsvecka 38.

I Studie II och IV analyserades blodprover från 53 kvinnor med redan diagnostiserad preeklampsi och 58 friska gravida kontroller i slutet av graviditeten. S100B och NSE var förhöjda även bland dessa kvinnor och för S100B korrelerade förekomst av ögonflimmer till ökade nivåer av S100B. I studie IV analyserades även nivåer av S100B och NSE i samma grupp av kvinnor ett år efter graviditetens avslut. Nivåerna av S100B och NSE visade sig då vara fortsatt förhöjda om än till en mindre grad.

I Studie V undersöktes effekten av experimentell preeklampsi och magnesiumsulfatbehandling på nivåerna av S100B och NSE i en råttmodell. Nivåerna av S100B och NSE skiljde sig inte mellan grupperna även om det fanns en tendens till högre nivåer av S100B hos råttor med experimentell preeklampsi jämfört med normal graviditet. Efter inducerad kramp hade friska råttor högre nivåer av S100B jämfört med gruppen utan kramp. Nivåer av NSE skiljde sig inte hos råttor med eller utan kramp. Efter kramp fans en tendens till högre koncentrationer av NSE hos råttor med experimentell preeklampsi jämfört med normal graviditet. Efter kramper hade råttor med experimentell preeklampsi och magnesiumsulfatbehandling högre nivåer av både S100B och NSE jämfört med råttor med experimentell preeklampsi utan behandling och råttor med normal graviditet.

Sammanfattningsvis har våra studier visat att hjärnskademarkörerna S100B och NSE är förhöjda vid preeklampsi jämfört med normal graviditet och att detta sker redan före tidpunkten för diagnos. Vi har vidare visat att högre nivåer av S100B korrelerar till förekomst av ögonflimmer hos kvinnor med preeklampsi. Förhöjda nivåer av båda markörerna kvarstår ett år efter graviditet hos kvinnor med tidigare preeklampsi jämfört med kvinnor med tidigare normal graviditet. I en djurmodell har råttor med experimentell preeklampsi högre nivåer av båda hjärnskademarkörerna i blodet vid behandling med magnesiumsulfat. Dessa fynd indikerar att hjärnan och/eller blod hjärnbarriären är påverkad hos kvinnor med preeklampsi före, under och efter diagnos. S100B och NSE är potentiella markörer att studera vidare för att i framtiden kanske kunna användas i kliniskt bruk som hjälpmedel att avgöra vilka kvinnor som har högra risk för komplikationer i hjärnan under och efter graviditet. Detta skulle isåfall vara en unik möjlighet att få insikt i vad som händer i hjärnan före, under och efter påverkan av havandeskapsförgiftning. Vidare studier krävs för att förstå rollen av hjärnskademarkörer i preeklampsi och om de kan hjälpa oss att förutsäga prognos på kort eller lång sikt hos kvinnor med preeklampsi.

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