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PLACENTAL HISTOPATHOLOGY IN PREECLAMPSIA AND OUTCOME OF THE OFFSPRING

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Till minne av min far

ABSTRACT

Preeclampsia (PE) is a major cause of maternal and fetal morbidity and mortality. The maternal symptoms are diverse and the neonates are often born premature and growth-restricted. Today the survival of premature infants has increased, but the neonatal complications including morbidity and long-term developmental deficits are still common.

The etiology and pathophysiological mechanisms in PE are still not known, but it seems as if a central part of the pathogenesis is associated with an unsuccessful implantation of the placenta into the uterus. The only cure is to deliver the mother, which is often a difficult decision in regard to prematurity of the fetus, when the disease has begun early in pregnancy.

In our first and second studies, we examined the placental pathology in relation to the maternal symptoms and severity of disease. The first study showed that there was a correlation between the severity of symptoms and the placental pathology. Further, the pathological picture was similar in mild and severe PE, but differed in relation to controls. To summarize, mild PE seems to be part of the PE spectrum, and not a normal physiological development of pregnancy, in contrast to what has previously been claimed.

The second study showed that placental pathology differed in severe PE with and without HELLP syndrome (hemolysis, elevated liver enzymes and low platelets), which is a disease regarded as a PE subtype, although the clinical picture is different from classical PE. This indicates that other mechanisms might be involved in the HELLP syndrome.

In the third and fourth studies, we examined the placental pathology in relation to perinatal, neonatal and childhood outcome. In the third study, we investigated the relation between placental pathology and perinatal and neonatal outcome in a cohort of PE patients and found that placental pathology was associated with adverse outcome. In the fourth study, in which we studied infants born extremely premature, we also found correlations between placental pathology and perinatal and neonatal outcome.

In the fourth study, we also explored possible relations between placental pathology and neurologic and developmental outcome of the child at the age of 2.5 years. We found a significant association between placental infarction and cerebral palsy (CP), and tendencies between several pathological findings and developmental outcome.

Overall, we have shown that the underlying pathologies in mild and severe PE probably are similar, whereas HELLP syndrome might have a different etiology. In addition, we have found associations between placental pathology and outcome of the offspring.

LIST OF PUBLICATIONS

I. **Marie-Therese Vinnars**, Josefine Nasiell, Sam Ghazi, Magnus Westgren, Nikos Papadogiannakis.
The severity of clinical manifestations in preeclampsia correlates with the amount of placental infarction.
ACTA Obstet Gynecol Scand. 2011;90:19-25.

II. **Marie-Therese Vinnars**, Liliane CD Wijnaendts, Magnus Westgren, Annemieke C Bolte, Nikos Papadogiannakis, Josefine Nasiell.
Severe preeclampsia with and without HELLP differ with regard to placental pathology.
Hypertension. 2008;51:1295-9.

III. **Marie-Therese Vinnars**, Josefine Nasiell, Gerd Holmström, Mikael Norman, Magnus Westgren, Nikos Papadogiannakis.
Placental pathology and neonatal outcome in preeclampsia: a large cohort study.
Submitted.

IV. **Marie-Therese Vinnars**, Nikos Papadogiannakis, Josefine Nasiell, Gerd Holmström, Brigitte Vollmer, Magnus Westgren.
Placental pathology in relation to neonatal and development outcome at 2.5 years of age in an extremely premature population: a prospective cohort study.
Submitted.

CONTENTS

LIST OF ABBREVIATIONS

AEDF	Absent end diastolic flow
AGA	Appropriate for gestational age
ALAT	Alanine aminotransferase
ANCOVA	Analysis of covariance
ASAT	Aspartate aminotransferase
BMI	Body mass index
BPD	Bronchopulmonary dysplasia
CI	Confidence interval
CP	Cerebral palsy
DNA	Deoxyribonucleic acid
DM	Diabetes mellitus
EXPRESS	Extremely preterm infants in Sweden study
HELLP	Hemolysis, elevated liver enzymes and low platelets
HLA	Human lymphocyte antigen
IUFD	Intrauterine fetal death
IVF	In vitro fertilization
IVH	Intraventricular hemorrhage
MFR	Medical Birth Registry
NEC	Necrotizing enterocolitis
NK	Natural killer
OR	Odds ratio
PE	Preeclampsia
PI	Pulsatile index
PNQ	Perinatal Quality Registry
PPROM	Preterm premature rupture of the membranes
PVL	Periventricular leucomalacia
RA	Rheumatoid arthritis
REDF	Reversed end diastolic flow
RNA	Ribonucleic acid
ROP	Retinopathy of prematurity
SD	Standard deviation
sFlt-1	Soluble vascular endothelial growth factor receptor-1
SGA	Small for gestational age

1 INTRODUCTION

Papyrus documents from around 2000 B.C. that have been found to describe gynecological disorders and contraceptives at that time are possibly also the first documentation of eclampsia, described as a suffering from the teeth and inability to open the mouth and if affecting the pubic region as far as the clavicles and the buttocks, indicating an incurable disease.[1, 2]

Eclampsia is a state of convulsions with a mortality rate as high as 14% in some countries, while the risk to succumb is much lower in other parts of the world.[3] Eclampsia progresses from preeclampsia (PE), a pregnancy-specific hypertensive disorder affecting 2-7% of pregnant women worldwide.[3, 4] Eclampsia and PE are not only affecting the mother, but are also associated with high morbidity and mortality in the infants.[3, 4]

In the year 2000, the United Nations set up 10 Millennium Development Goals, two of which were to lower maternal and child mortality.[5] Maternal mortality varies greatly depending on geographical area. Women living in middle- and high-income countries have a pregnancy-related mortality risk of one in 4 000 – 20 000, whereas the corresponding risk in some low-income countries is reported as high as up to one in 15.[6, 7] In African and Asian countries, the most common causes of maternal deaths related to pregnancy are hemorrhage, infections and hypertensive disorders.[8]

In Sweden, and in many other countries, the maternal and reproductive care is well developed and regular antenatal controls by midwives contribute to an earlier identification of women suffering from PE, thus making it possible to intervene when needed. Curative treatment is however not available, but if necessary, labor can be induced. Conservative management consists of administration of antihypertensive drugs and magnesium sulfate, and/or expectancy.[9] In spite of the extensive prenatal care, hypertensive disorders are the greatest single cause of maternal deaths in high-income countries, accounting for one in six of maternal deaths.[8]

Depending on factors such as gestational age and severity of disease, up to 67% of PE infants are reported to be born prematurely.[4] In high-income countries, the survival rates of very preterm birth infants have increased, but neonatal morbidities and delayed development are common problems with decreasing gestational age.[10, 11]

Today, 4000 years after the ancient Egypt, women and infants still succumb and suffer as a consequence of eclampsia and PE and the etiology of the disease is still unknown.[4] It is essential to elucidate the pathophysiological mechanisms in PE, as part of an attempt to find strategies in preventing the development and progression of the disease and in treating and possibly also curing the women and infants affected.

2 BACKGROUND

2.1 THE BEGINNING OF LIFE

The embryonic development begins when an oocyte and spermatocyte fuse and form a zygote. The zygote travels towards the uterine cavity and meanwhile, cell division occurs. By day four, the zygote has become a 32-cell mass, looking like a mulberry and called morula (latin word for mulberry). The morula reaches the uterine cavity by day six and is now called a blastocyst, which will implant into the uterine wall.[12]

In a non-pregnant woman, the spiral arteries in the uterine wall have each month grown longer and finally collapse, giving rise to a menstruation. When an embryo implants, the arteries are transformed so that they can satisfy the infant's need for nutrients and oxygen.[12]

2.2 THE PLACENTA

2.2.1 Development

The blastocyst consists of trophoblast cells, which will form the fetal side of the placenta, and embryoblast cells, which will form the fetus. As the blastocyst adheres to the uterine wall, some of the trophoblasts start to proliferate, lose their cell membranes and fuse. These, so called syncytiotrophoblasts, expand and finally surround the entire embryo. Within the syncytiotrophoblasts, trophoblastic lacunae form, see figure 1.[12]

As a response to the implantation of the blastocyst, the endometrial stroma becomes highly vascularized and thickened. The endometrium has developed into a decidua, which will form the maternal side of the placenta. The capillaries of the maternal spiral arteries become maternal sinusoids, which anastomose with the trophoblastic lacunae and become filled with maternal blood, see figure 1. This is the beginning of the uteroplacental circulation.[12]
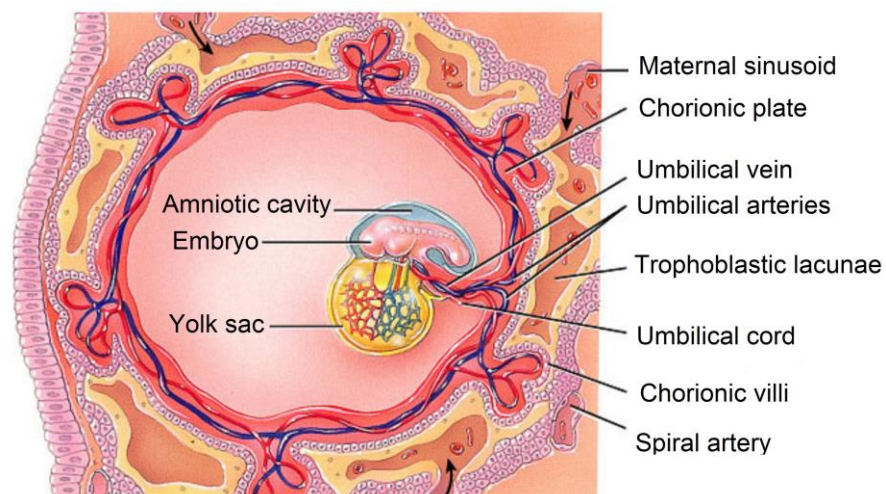

Figure 1. An implanted blastocyst. Reprinted with permission from John Wiley and Sons, Inc.

The trophoblasts that did not become syncytiotrophoblasts are called cytotrophoblasts. Covered by syncytiotrophoblasts they are forming several extensions, called villi, which grow into the blood filled lacunae. Later on, embryonic cells will form fetal blood vessels that will be found within those villi. The lacunae have now fused together and become one space, into which the villous trees expand. Hence, the room is called the intervillous space. The layer of cytotrophoblasts and syncytiotrophoblasts between the fetal blood vessels in the villi and the maternal blood in the intervillous space make up the placental bed barrier, and is called the syncytiotrophoblastic membrane. The development of the villous structure will continue during the entire pregnancy. Villi at different gestational weeks can be seen in figure 3A and C.[12, 13]

In a normal pregnancy, the spiral arteries become transformed. The vasoreactive endothelial and smooth muscle cells of the artery walls are exchanged with trophoblast cells that do not respond to vasoconstrictors and hence, those arteries are constantly dilated and unconditionally filling the intervillous space with maternal blood. Oxygen and nutrients thereafter diffuse through the placental bed barrier and are further transported within the fetal veins which are located in the villi. Merging together they form one umbilical vein continuing towards the infant. In the other direction, two umbilical arteries transport (poorly oxygenated) fetal blood from the infant through the umbilical cord towards the placenta.[12-14]

2.2.2 Gross anatomy

The placenta is made out of both maternal and fetal tissue, as is described above. It can be divided into the placental disk, the membranes (amnion and chorion) and the umbilical cord, see figure 2. The placental disk consists of the decidua, where the maternal blood vessels (spiral arteries) are; and the chorionic plate, which is on the fetal side of the placenta. The space between the maternal decidua and the fetal chorionic plate is the blood filled intervillous space, in which fetal chorionic villi are located. The umbilical cord is normally inserted on the chorionic plate. The fetal side of the placenta is covered by an amniotic and a chorionic membrane which also surround the entire fetus.[13]
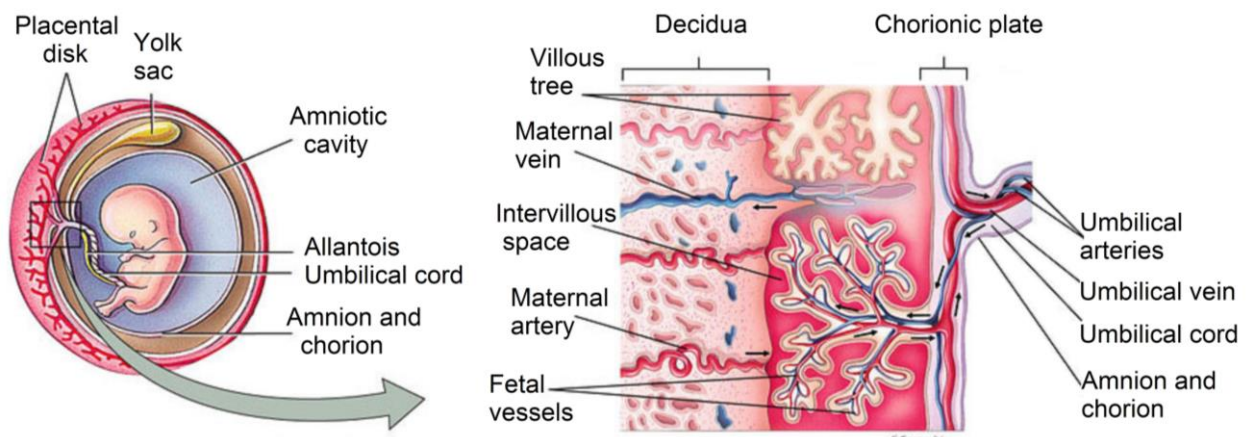

Figure 2. Placental structure. Reprinted with permission from John Wiley and Sons, Inc.

2.2.3 Placental pathology

The focus of this thesis is on placental pathology that is related to PE, prematurity and adverse fetal outcome. In general, much of the pathology discussed in this thesis is related to perfusion defects.

2.2.3.1 *Pathology often seen in PE*

2.2.3.1.1 Decidual arteriopathy

The invasion of trophoblasts into the spiral arteries does not occur sufficiently in all pregnancies and as a consequence the spiral arteries continue to constrict in response to vasoconstrictors.[14-18] The corresponding histopathological findings are hypertrophy of the smooth muscle of the artery wall and hyperplasia of the endothelium. Later on, fibrinoid necrosis of the wall, luminal thrombosis and atherosis of the artery walls are present.[19] In acute atherosis foamy macrophages are localized within the spiral artery wall.[13] Some of the signs in decidual arteriopathy can be seen in figure 4A and B.

Absent transformation of the spiral arteries is primarily associated with PE and fetal growth restriction[14, 19], but some studies have more recently also showed associations with spontaneous abortions in second trimester and preterm labor.[20, 21] Some years ago, Redline et al[22] showed an increased risk for cerebral palsy (CP) in cases with acute atherosis.

Acute atherosis resembles arteriosclerosis and is also related to later cardiovascular disease of both mother and infant. PE and cardiovascular disease have many common risk factors and pregnancy could be regarded as a stress test of the mother's susceptibility for cardiovascular disease.[23]

2.2.3.1.2 Placental infarctions

The villi, and in continuation the infant, are dependent on the maternal vessels' supply with oxygen. As a consequence of occlusion of maternal arteries the adjacent villi become infarcted. Infarctions can be seen grossly, especially if they are of older age, becoming pale, and if they are extensive. Histologically they represent typical ischemic necrosis of placental parenchyma, i.e. chorionic villi. Initially, the intervillous space within the ischemic area is reduced, and villi tend to aggregate and are congested with dilated capillaries. Further on, pyknosis and karyorrhexis can be seen. After a longer time the villous structure looks like a shadow of the former villi, sometimes referred to as ghost villi, see figure 5B.[13, 24]

Placental villous infarctions of greater extent are more common in hypoxia, fetal growth restriction, intrauterine fetal death (IUFD) and PE.[13] Ischemic changes in the villi have also been associated with periventricular leucomalacia (PVL)[25, 26] and macroscopic placental infarctions have been related to CP in the infant.[27, 28] A study by van Vliet et al[29], comparing infants affected by chorioamnionitis and placental underperfusion, among other signs including infarctions, found a lower developmental score at 2 years of age in the underperfusion-group.[29] Another similar study[30] did not find any association, but had a smaller material, shorter duration of follow-up and did not adjust for confounding factors.

Infarctions involving <5% of the tissue can be found in 25% of normal term placentas. Infarctions of small extent and especially if located in the periphery are not associated with adverse outcome.[13, 19]

2.2.3.1.3 Intervillous thrombosis

The untransformed spiral arteries seen especially in PE and fetal growth restriction are proposed to be causing a disruptive turbulence of the blood flow within the intervillous space.[31, 32] Here, so called intervillous thromboses can be seen macroscopically, but can be mistaken for infarctions without histological confirmation.[13, 33] Microscopically, they are characterized by fibrin lamellae mixed with fetal and maternal erythrocytes. See figure 6A. Later on, the blood cells degenerate and the laminations become more compact. Especially in PE and fetal growth restriction, the intervillous thromboses can be found.[13]

Hutchinson et al[34] performed a study on a placenta perfusion model, in which they altered the blood flow to the placenta and examined the impact on the villi. They found that an alternation in the hemodynamics, as in PE, had a destructive effect on the villi and resulted in a release of factors associated with endothelial disruption, hence suggesting a link between changes in blood flow to the placenta and maternal symptoms.

2.2.3.1.4 Accelerated villous maturation

Reduced blood supply to the placenta can lead to an acceleration of the placental villi. The villi become smaller, involve less stroma and the so-called syncytiovascular membrane, separating the fetal and maternal blood, becomes thinner. This morphology is very similar to the appearance of a term placental villi. See figure 3A-C. The phenomenon is more common in preterm birth, PE and fetal growth restriction, but can also be seen in placentas from smoking mothers.[13, 24]

2.2.3.1.5 Abnormal placental weight

The placental weight is often affected by a reduced uteroplacental blood flow. A low placental weight in relation to its gestational age is related to both a low birth weight and presence of placental infarctions that can also be associated with an adverse blood flow in the placenta.[13] A small placenta in relation to its gestational age is common in hypertension, PE, diabetic vasculopathy and fetal growth restriction. Small placentas can also be observed in association with chromosomal abnormalities.[13, 24]

However, more recently, also a higher frequency of large placentas in relation to gestational age has been reported in a population of term PE.[35] A large placenta is more common in maternal diabetes mellitus (DM), fetal hydrops, hemoglobinopathy, Rh incompatibility, acute and chronic infection, maternal anemia and overgrowth syndromes. The placenta can also be heavier as a result of a retroplacental hematoma or maternal fluid administration during cesarean section.[13]

2.2.3.1.6 Abruptio placentae

Abruption of the placenta is a premature separation of the implanted placenta from the uterine wall due to the rupture of maternal arteries. The cause is unknown, but risk factors such as PE, hypertension, smoking, cocaine use, chorioamnionitis and premature rupture of the membranes have been identified.[13]

Placental abruption could cause abdominal pain and is a leading cause of vaginal bleeding in the second half of pregnancy, but can also occur without any bleeding symptoms. The abruption can be partial or total and depending on the extent, the consequences differ greatly. A total abruption can cause fetal death, whereas a partial abruption could become chronic and result in no obvious symptoms or sequelae.[36]

There is often a discrepancy between the obstetric and histopathologic diagnosis of abruption. The clinically diagnosed abruption is more often acute and is only verified by the pathologist in 30% of the cases. On the contrary, a pathological finding of a retroplacental hematoma, indicating an abruption, is only coexisting with a clinical diagnosis in 35% of the cases. Whereas an acute abruption could be histopathologically undetected, chronic abruptions not only give rise to organized blood clots and craters of the placental tissue, but also to additional histological findings such as hemosiderin deposits or signs of reduced placental perfusion.[13, 36] See figure 7A.

2.2.3.2 Some other common pathology features

2.2.3.2.1 Fetal thrombosis

Fetal thromboses are arterial or venous and are localized either in the vessels of the umbilical cord, the chorionic plate or the villous tree. A careful macroscopical examination may identify the thrombosis, which in the acute phase can be seen histologically as a laminated thrombosis attached to the endothelial surface. If chronic, the thrombosis often has undergone a transformation making the vessel wall organized and thickened. See figure 6B. The etiology of fetal vascular thrombosis is probably multifactorial.[13, 19, 37, 38]

Fetal thrombosis can result in an embolic event both in fetal and placental direction, depending on the type of vessel. In the presence of an arterial thrombosis, the event could occur in the villi, resulting in atrophy of the villi and, if extensive, result in growth restriction or fetal death. On the other hand, in case of a venous thrombosis, the embolic event could happen in the infant and have an impact on the fetal brain, viscera or upper extremity.[13, 24]

Fetal thromboses have been related to IUFD, fetal growth restriction, neonatal encephalopathy and delayed development.[26, 39-44] Some studies[45, 46] have not seen any relation between fetal thrombotic pathology and neonatal brain injury, but they have been smaller and not taken confounding factors into account. However, although studying a limited number of patients, Elbers et al[47] found a high incidence of fetal thrombosis in a group of patients with neonatal stroke.

2.2.3.2.2 Chorioamnionitis

Acute chorioamnionitis is the most frequent placental finding in infants born before gestational age 32 with a decreasing prevalence with advancing gestational age. It is an inflammatory response localized to the membranes and the chorionic plate. Most often microbes ascending from the urogenital tract are responsible for the inflammatory response. The symptoms of acute chorioamnionitis are maternal fever, leukocytosis, prolonged rupture of the membranes, uterine tenderness, foul smelling amniotic fluid and fetal tachycardia. A so called silent chorioamnionitis can be diagnosed histologically when none of these symptoms is present.[13, 48, 49]

Histologically, maternal neutrophils are found in the membranes, the umbilical cord, the chorionic plate and/or the subchorionic space. Fetal neutrophils may be observed in the fetal vessels of the chorionic plate or umbilical cord. Macroscopically, the membranes may be thickened and have a yellowish color because of the inflammatory exsudate.[13, 48, 49] See figure 7B.

Acute chorioamnionitis is related to placental abruption, preterm birth and adverse perinatal outcome.[36, 37, 48] It has been further associated to pulmonary disorders, sepsis and cerebral complications in the neonate, but studies have shown discrepant results.[22, 26, 43-45, 50-64] A likely underlying mechanism could be formation of thrombosis in the fetal vessels that are damaged from the inflammatory cells. Those could hypothetically embolize via the umbilical cord to the fetal lung and brain.[37]
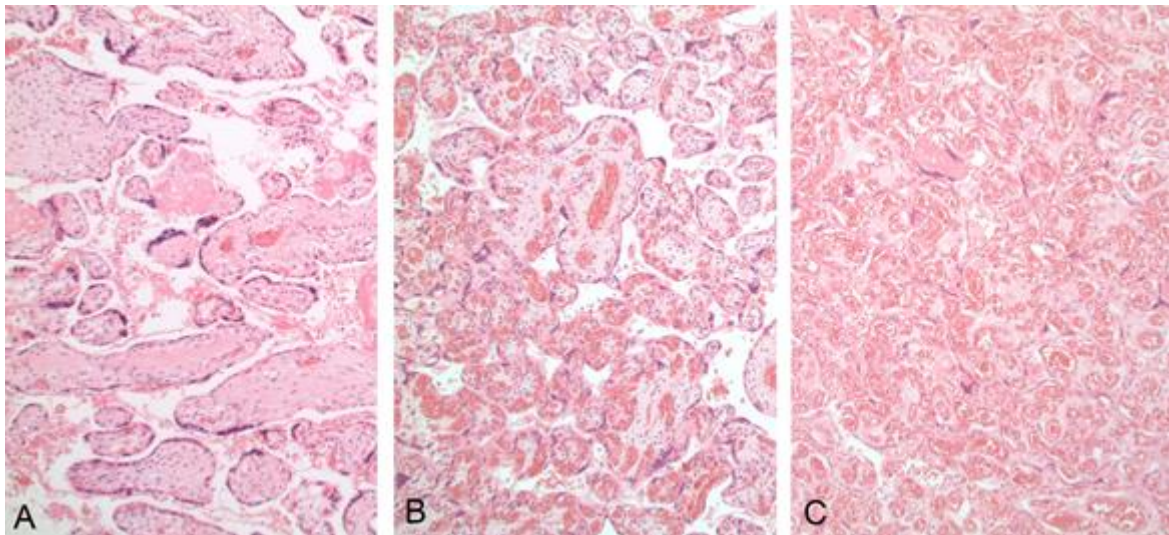
Figure 3A. Normal appearance of the villi in gestational week 29. **B.** *Accelerated villous maturation* in gestational week 29. **C.** Normal appearance of villi at term.
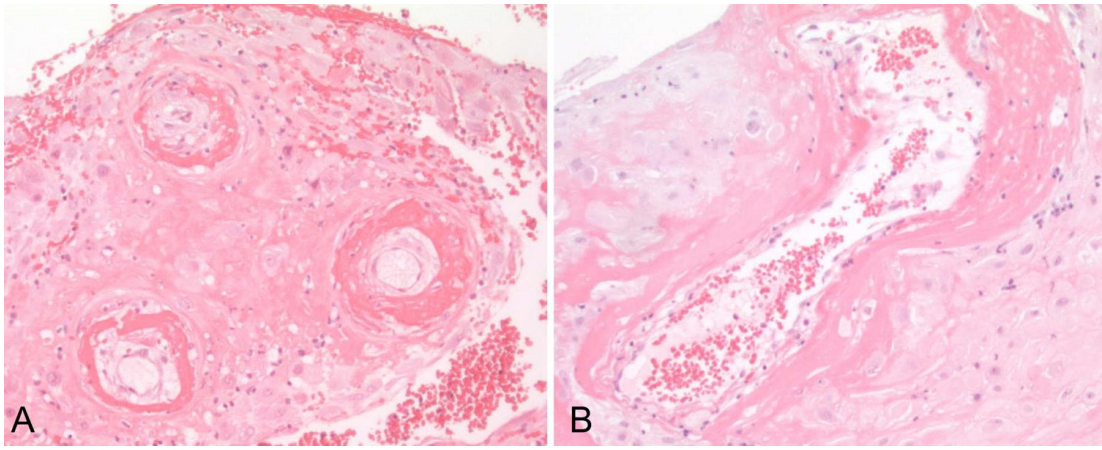
Figure 4A and B. *Decidual arteriopathy* is seen in the maternal blood vessels as dilated lumen, fibrinoid necrosis of the vessel wall and presence of foamy macrophages.
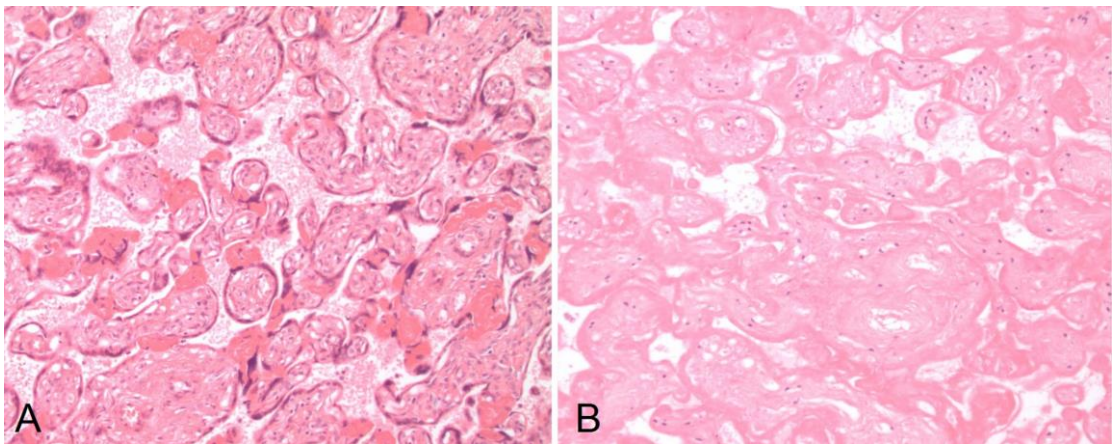
Figure 5A and B. *Placental infarction* is seen in right picture, normal villi from the same placenta in the left. This infarction is of older age, composed of so called ghost villi.
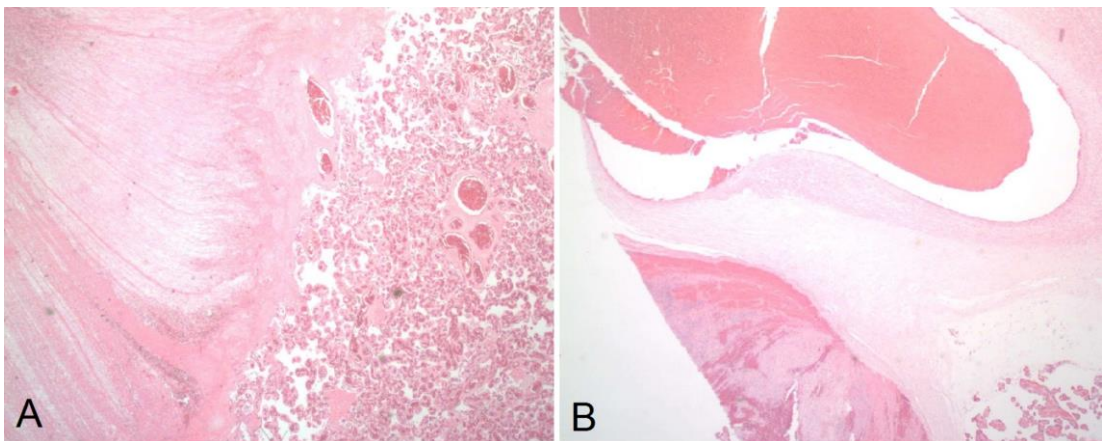
Figure **6A**. *Intervillous thrombosis* can be seen to the left, normal term placenta to the right. The thrombus is of older age, as fibrin lamination is present, and contains both maternal and fetal erythrocytes. **6B.** Dilated chorionic plate vessels with fetal thromboses. The vessel in the upper part of the picture shows so-called endothelial cushion with intimal mural fibrin deposition. The vessel in the lower part shows older, laminated fibrin thrombus with occlusion of the lumen.
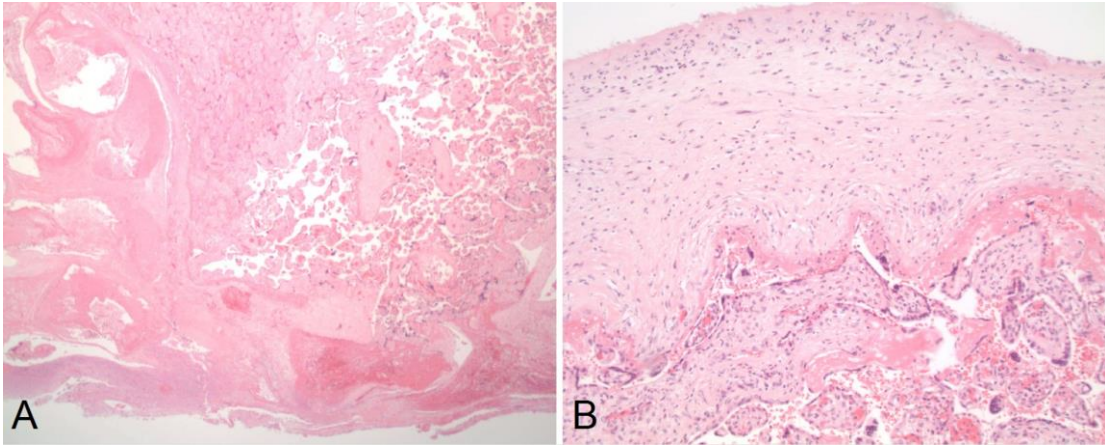
Figure 7A. *Placental abruption* with older retroplacental clot, creating crater formation (left) and adjacent infarction (upper left). **B.** *Chorioamnionitis* with neutrophils in the subchorionic space as well as in chorion and amnion layers.

2.3 PREECLAMPSIA (PE)

2.3.1 Definition

PE is a pregnancy specific syndrome mainly based on presence of hypertension and proteinuria. According to current criteria, PE is diagnosed in the presence of a systolic blood pressure of 140 mmHg or higher or a diastolic of 90 mmHg or higher on two occasions at least six hours apart, and proteinuria, defined as an excretion of at least 0.3 gram protein during 24 hours.[65, 66] The symptoms can occur at any time after 20 weeks gestation, but early debut is usually more serious, and suggestions have been made to divide PE into an early- and late-onset syndrome.[67] Placental pathology is reported to be more frequent and severe in early gestational age.[68]

2.3.1.1 Severe PE

PE is clinically divided into a mild and a severe form. Severe PE is diagnosed in the presence of even higher blood pressure, proteinuria, fetal growth restriction or maternal complications. Presence of symptoms included in table 1 indicates a severe form of the syndrome.[65] If not treated, PE can progress to eclampsia, defined as the development of convulsions or coma in PE patients.[3]

Table 1. Indication of severe PE[65]
Systolic blood pressure ≥ 160 mmHg
Diastolic blood pressure ≥ 110 mmHg
Proteinuria ≥ 5 g/24 hour
Oliguria <500 mL/24 hour
Cerebral or visual disturbances
Pulmonary edema or cyanosis
Epigastric or right upper-quadrant pain
Impaired liver function
Trombocytopenia
Fetal growth restriction

2.3.1.2 HELLP syndrome

Another severe complication of PE is HELLP syndrome (acronym for hemolysis, elevated liver enzymes and low platelets). In addition to hypertension and proteinuria, typical symptoms in HELLP syndrome are malaise, upper quadrant tenderness, nausea and vomiting. Diagnosis of HELLP is based on laboratory findings and subjective symptoms such as epigastric pain. The presence of hemolysis is best defined as low haptoglobin levels, elevated liver enzymes are often defined as an elevation of alanine aminotransferas (ALAT) and aspartate aminotransferas (ASAT) above 2-3 standard deviations (SD) above the mean and thrombocytopenia defined as platelets ≤ 100.000 /µl. Hypertension and proteinuria is absent in 10-15% of women with HELLP syndrome, suggesting a possible heterogeneity within this disease entity.[4, 69, 70]

2.3.2 Epidemiology

PE affects approximately 2-7% of pregnant women worldwide and is associated with both maternal and fetal mortality and morbidity. Although outcome is better in the developed world, PE accounts for 15-20% of maternal mortality.[4] Along with

hemorrhage and infection, hypertension is the major maternal complication in pregnancy.[71] [72] In three quarter of the cases, the syndrome starts near term, but it can also begin as early as in gestational week 20. Preterm delivery is reported in 15-67% and fetal growth restriction in 10-25% of PE pregnancies.[4]

2.3.3 Pathophysiology

The etiology of PE is still unknown, although it is established that the presence of a placenta is necessary in the development of PE. It is also generally accepted that a normal pregnancy is achieved through a successful implantation of the placenta into the uterine wall and that the placental implantation in PE seems to be incomplete.[21]

2.3.3.1 The disease of theories

As the speculations have been many, PE has been described as the disease of theories. One of the leading theories at the moment is that there is a maternal immunological response to the fetal immune system, resulting in abnormal transformation of the spiral arteries, giving rise to some of the pathology described above. See figure 8A-B. The absence of transformed spiral arteries leads to a high-resistance blood flow to the placenta. The turbulence of the blood flow and hypoxia in the placenta then give rise to a destruction of placental tissue and release of factors, such as soluble vascular endothelial growth factor receptor-1 (sFlt-1), syncytiotrophoblast membrane microparticles, fetal ribonucleic acid (RNA) and deoxyribonucleic acid (DNA), which might injure maternal endothelium and be responsible for the maternal symptoms.[4, 21, 73-76] In addition, a breakage of the placental bed barrier has also been shown in PE patients.[77]
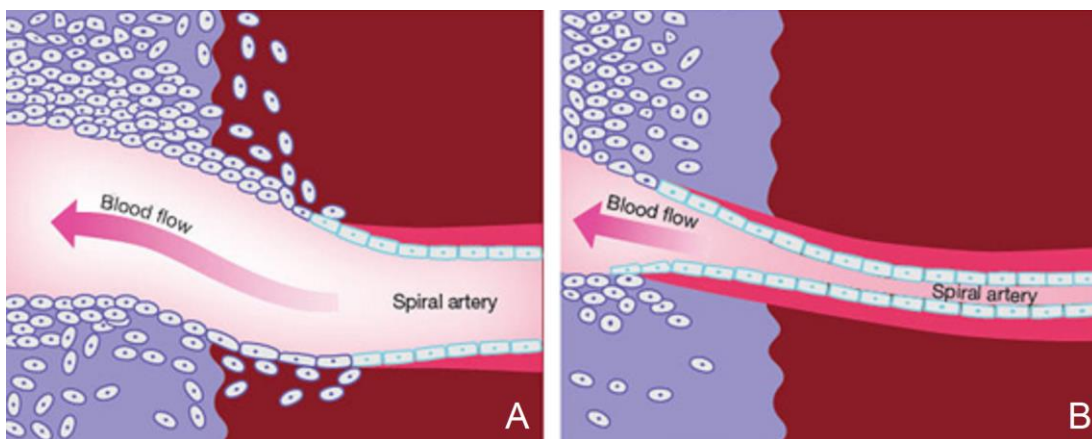
Figure 8A: Normal trophoblast invasion into the spiral arteries, resulting in a low-resistance blood flow. **B:** Incomplete transformation of the spiral arteries, seen in PE, resulting in a high-resistance blood flow. Reprinted with permission from Eunice Kennedy Shriver NICHD, National Institutes of Health, U.S.

2.3.3.2 Immunological considerations

The reason for the failed implantation in PE is unknown, but it has been speculated whether PE is a type of rejection of the fetus, similar to the rejection of a transplanted organ. Incomplete placental implantation has recently also been associated with spontaneous abortions.[20, 21] Some researchers have also speculated whether PE might

be an autoimmune disorder.[78] Inevitably, the fetal cells carry genetic material both from the mother (that is recognizable) and from the father (that is foreign).[79]

After mating, an inflammatory response can be seen in the female genital tract, giving an opportunity for the maternal immune system to process the paternal antigens. This could direct the maternal immunological response towards a tolerant T helper 2-response[4], which has been found to be related to a successful pregnancy.[80] On the other hand, a T helper 1-response, which is less tolerant, is more common in PE, but also in miscarriages.[80] However, T- and B-cells are not common populations in the placenta and the role of NK (natural killer) cells has also been discussed.[81, 82] Also, it has been speculated whether NK cells could mediate the change in T helper type.[83]

Several risk factors (see table 2) have been identified that would support the idea of an adaptation and development of a tolerance towards the paternal antigens. Robillard et al[84, 85] found that the time of cohabitation before conception was inversely related to the risk of pregnancy induced hypertension. Also, others have shown an increased risk for PE after short cohabitation and after use of barrier contraception[86] as well as a decreased risk for PE if the coupled had practiced oral sex before the pregnancy.[87] The risk for PE is also higher in the first pregnancy than in the proceeding and both previous abortions and healthy pregnancies are protective against PE.[88-91] However, with change of partner, the protective effect of a previous pregnancy or abortion is lost.[88, 92]

Studies on donated ova and sperm cells also support this theory. A study by Salha et al[93] showed higher frequencies of PE in patients who had received sperm (18%), ovum (16%) or embryo (25%) donation than in a control group (0-3.7%). Also, they found more PE in women who had undergone in vitro fertilization (IVF) if the sperm cells came from a donor (18%) than if they came from their partner (0%). Wang et al[79] hypothesized that couples in need of surgical obtainment of sperm cells might have an increased risk of PE as the female had not been exposed to the sperm cells prior to conception. They found that those couples had a threefold risk for PE compared with couples where IVF was performed with ejaculated sperm cells.

2.3.3.3 Maternal PE

Why an abnormal placental implantation results in PE in some women and in fetal growth restriction, spontaneous abortion or preterm prelabor rupture of the membranes in others is not clear.[21] One can speculate whether those obstetrical issues are different sides of the same coin. However, it has been proposed that defective implantation is linked to the maternal symptoms in PE by a metabolic syndrome in the mother, which is not present in fetal growth restriction without PE.[94]

Maternal obesity[89, 95-99], DM[4, 95], insulin resistance[100], hypertension and renal disease[95, 98, 101, 102], thrombophilia[103] and rheumatoid arthritis (RA)[104] have been identified as risk factors for PE (see table 2). With the exception of smoking, which decreases the risk for PE[89, 95, 98], cardiovascular disease and PE share many risk factors.[23] Also, PE increases the risk for later cardiovascular disease.[105-107]

The profile of maternal risk factors outlined above has led some authors to propose a division of PE into a maternal and a placental type of the disease. In the maternal form, a mother with many cardiovascular risk factors could develop PE as a response to a normal pregnancy with corresponding degree of inflammation. Conversely, in the placental form, a mother without any risk factors could develop PE as a response to a malfunctioning placenta with a higher degree of inflammation than in a normal pregnancy.[74]

Table 2. Risk factors for PE

First pregnancy[4, 91]
Limited sperm exposure[84, 86, 87]
Donation of ovum, sperm or embryo[79]
Obesity[89, 95-99]
DM[89, 95]
Insulin resistance[100]
Hypertension and renal disease[95, 98, 101, 102]
RA[104]
Non-smoking[89, 95, 98]
Multifetal gestation[108, 109]
Heredity[110-113]
Previous PE[114]
Thrombophilia[103]

Many substances have been found to be released from the placenta into the maternal circulation.[4] The amount of those might also influence the presence of maternal symptoms, which could explain why women with multiple gestation[108, 109] have a higher risk of PE.

2.3.3.4 Genetic factors

Previous studies have also shown that genetic components in some way contribute to the development of PE.[2, 110, 112, 113, 115] In total, over 170 genes have been related to PE and HELLP syndrome, but no definitive conclusions have been drawn, probably because the genetic mechanisms in PE are complex and diverse.[115] However, it has been shown that a woman who becomes pregnant with a man who has fathered a previous PE pregnancy in another woman has an increased risk for PE.[116] Studies have also shown that both males and females, whose mothers had PE, have an increased risk for PE in their pregnancies.[111, 117]

What effect the genes have on PE is unknown, but it is likely that they are involved in the interface between the mother and fetus.[2] In the placenta, the fetal trophoblasts that invade the maternal tissue meet cells of the maternal immune system, amongst others NK cells, which recognize fetal polymorphic human lymphocyte antigens-C (HLA-C). In vivo studies have shown that certain combinations of HLA-C haplotypes and receptors on the NK cells are associated with an increased risk for PE[74]. Hiby et al[118] concluded that some combinations of paternal and maternal innate immunity genes were unfavorable for placentation. This might explain part of the disease.

2.3.4 Doppler ultrasound

An abnormal transformation of the spiral arteries most probably underlies the circulatory dysfunction seen in PE and fetal growth restriction. Since the 1980's Doppler ultrasound measurements have been increasingly used to obtain additional information regarding the impact of PE on the fetus and in the screening of maternal hypertension.[119-124]

Today, it is known that abnormal umbilical artery Doppler findings are associated with fetal hypoxia, fetal acidosis and adverse perinatal outcome. Measurement of the blood flow in the umbilical artery is now used in order to improve perinatal outcome in pregnancies complicated by PE and fetal growth restriction.[125] Normally, at approximately twelve weeks of gestation not only a systolic blood flow is present in the umbilical artery, but also a diastolic. From then on, the diastolic blood flow increases, but in the presence of malfunctioning villi, an increased resistance in the placenta does not permit this increase. As a result, the diastolic component decreases, become absent or even worse: reverse. The abnormal blood flow is then classified as absent end diastolic flow (AEDF) or reversed end diastolic flow (REDF).[126]

Other vessels have also been investigated.[127-131] Impaired trophoblast invasion into the spiral arteries has been related to abnormal uterine artery blood flow.[132, 133] A normal trophoblastic invasion results in a physiological decrease of resistance of the uterine artery. In the absence of trophoblast invasion the decrease of resistance does not occur. Hence, measurement of blood flow in the uterine artery is used in order to find pregnancies complicated by fetal growth restriction and PE. Abnormal blood flow of the uterine artery has also been related to IUFD and abruption.[127]

Nonetheless, the usage of uterine artery blood flow in screening for PE is not entirely reliable and several researchers have tried to find maternal serum markers in the screening for PE.[134, 135] None has been found to anticipate PE, but an early PE could be predicted with the use of both Doppler measurements and a combination of several biomarkers.[134] Apparently, screening of women with risk factors was actually less successful than screening of low-risk individuals.[134] This could indicate that women with cardiovascular risk factors were likely to develop a maternal PE, whereas women without risk factors developed a placental PE as a consequence of an impaired placentation.[4, 9, 134]

Some placental pathology has also been related to umbilical and uterine artery blood flow. Higher resistance in the umbilical artery has been related to fewer blood vessels in the villi [136, 137] and more placental infarction.[138-140] Also abnormal uterine artery blood flow has been associated with placental ischemia.[139]

2.3.5 Treatment for PE

There is no other curative therapy of PE than induction of labor, which is a difficult decision in earlier pregnancies. At term, both mild and severe PE should be managed by induction of labor [9, 141], but before gestational week 34 expectant monitoring benefits the child, if possible due to the clinical situation of the mother.[142]

If induction of labor is chosen, suggested routes of delivery depend on gestational age and fetal and cervical status.[9] If expectant monitoring is chosen, antihypertensive agents should be considered. No specific antihypertensive drug is preferred and the recommendation is to use one that the clinician is familiar with.[9] Magnesium sulfate should be administered in severe PE patients to reduce the risk of eclampsia and in patients who have already developed eclampsia. Further, low-dose aspirin has been shown to decrease the risk for PE, but the numbers needed to treat is large and this is only recommended in high risk patients.[9] More recently, studies have examined the effect of Heparine, which was shown to decrease the risks for PE and eclampsia, but as information on adverse outcome of the offspring is unavailable, more studies need to be performed.[143, 144] As some pathology in PE is similar to arteriosclerosis and those disorders share many risk factors, trials on statins are also conducted.[145]

2.3.6 Consequences of PE for the infant

PE-infants are often growth-restricted, have a higher risk for IUFD and are more often born premature, which is itself a great risk factor for adverse outcome, as the next section will outline.[9, 10, 74, 146] It seems as if PE-infants also need neonatal intensive care in a greater extent [147-149], but studies on neonatal morbidities have not been very convincing and often show contradicting results.[147, 150-156] However, PE might be protective against retinopathy of prematurity (ROP).[157] A couple of studies have showed a lower intelligence quote in PE children[155, 158], but others have shown that PE is not related to special needs.[159] Maybe, induction of labor also has an effect on the outcome of the infant.[160]

In adulthood, children born to mothers with PE are shown to have higher blood pressure and higher body mass index (BMI) in adulthood.[161, 162] The effect on body weight appears to be true also when excluding the effect of low birth weight, which can result in catch-up growth.[163]

22

2.4 PREMATURITY

2.4.1 Definition

Preterm birth is a delivery before 37 completed weeks of gestation. In addition, very preterm birth is defined as a birth before 32 gestational weeks and extremely preterm birth is a delivery before 28 gestational weeks.[164]

2.4.2 Etiology

Preterm birth can be divided into three categories. Firstly, about one third (30%) of the preterm births are caused by induction of labor, often because of fetal growth-restriction, fetal demise or maternal hypertension. The remaining two thirds are spontaneous preterm births, which are divided into spontaneous preterm labor (45% of preterm births) and preterm premature rupture of the membranes (PPROM) (25% of preterm births). A spontaneous preterm labor is defined as a preterm onset of regular contractions and change in cervical status, whereas PPROM is defined as spontaneous preterm rupture of the membranes at least one hour before onset of contractions.[71, 165]

The exact mechanisms behind spontaneous premature birth are unknown, and it is seen as a syndrome caused by several factors. Risk factors such as infection, inflammation, stress, uterine distension (as in multifetal gestation and in extremes in volume of amniotic fluid), low pre-pregnancy BMI, previous preterm birth, tobacco use, close interval between pregnancies and assisted reproduction (both in single and multiple pregnancies) have been identified.[165]

2.4.1 Epidemiology

The incidence of prematurity is around 12-13% in the United States and has risen during the last years.[165] The main contributing factor is an increase in induction because of fetal or maternal reasons, such as growth restriction and PE. An additional factor is the increasing use of assisted reproduction technology.[165] However, the incidence varies depending on geographic area[166] and in Sweden there were 5.5% premature deliveries during 2011.[167]

2.4.2 Consequences of prematurity for the infant

Infants born premature are at increased risk for morbidities such as bronchopulmonary dysplasia (BPD), ROP, intraventricular hemorrhage (IVH), PVL and necrotizing enterocolitis (NEC).[71] Those are described further in the following section.

Several research groups have studied children born preterm and compared today's outcome with survival and morbidity from previous years.[10, 11, 168-170] The survival rate is increasing, but some of the major morbidities are shown to be the same as previously.[10] The neurodevelopment is often lower[169, 170] and Moore et al[11] have shown an increase in developmental scoring with gestational age in infants born before gestational week 27. Nowadays, the incidence of CP is reported to be 7-14%.[11, 170]

2.5   THE OFFSPRING

2.5.1   The normal scenario

2.5.1.1   Intrauterine development

Human gestation is about nine months long and often divided into three periods, the trimesters. In week 3-8 during the first trimester the organs and systems are formed. With the exception of the heart and the vasculature systems, most organs are not functional at that time. In the second trimester the organs continue to develop, but some organs, such as the reproductive organs and the brain do not mature fully until years after birth. In the last trimester the lungs mature and the majority of the weight gain occurs.[12]

While living inside the maternal uterus, the infant is dependent on the maternal supply of oxygen and nutrients, but also on the maternal immune system's ability to allow the presence of another individual. Teratogens and microbial pathogens might also cross the placenta and affect the unborn child. Another important factor for the infant's well-being is the balance of the amount of amniotic fluid.[12]

From the start of the third trimester until term, the infant grows from less than 1 kilogram to about 3.5 kilograms. During gestation, the body proportions also change: at nine weeks the head is half the crown-rump length, but at birth it is a quarter of the same length.[12, 171]

At birth, it is essential that the infant starts breathing, which is stimulated by accumulation of CO_2 and lack of O_2, but also by the compression of the thorax during delivery. After birth, fluid in the neonatal lung is exchanged for air and soon the infant breathes regularly. In the delivery room, assessment of the newborn is important in order to identify infants in need of resuscitation. The acid-base status of the umbilical cord blood is examined. Additionally, Apgar scores are measured at 1, 5 and 10 minutes of age and parameters evaluated are heart rate, respiratory effort, muscle tone, reflex irritability and color. [71, 172]

2.5.1.2   Childhood development

As early as gestational week 20, the fetus starts to respond to auditory stimuli with a change in heart rate or by moving.[173] Apparently, it can also distinguish between different sounds such as language and music.[173] The newborn's preference of sound is voices, and then, especially the voice of the mother.[12, 173] During the first half year of life, the infant enjoys making noises and laughs and after a little more than a year, it starts to make specific sounds to the parents.[174]

Just as the sound preference is voices, the visual preference is faces.[174] During the first year of life, the child's visual acuity, ability to move the eyes together and to focus at different distances are improved and already at the age of about 4 years, adult level of visual acuity is reached.[174] Fine motor function develops together with vision, and at ten months of age, half of them use the pincer grip and at 1.5 years of age, they begin to draw with a crayon.[174] The limit age for walking is 18 months, which is when only

2.5% still have not learned to walk and should be examined further, although many of them will not have any underlying problem.

The first 5 years of life is the period when the child development occurs, with a remarkable improvement in motor function, language, hearing and visual development. However, preschool children do think that they are the center of the world, non-living objects are alive and have feelings, and that events are magic. Much of the cognitive development occurs at school age and although a small child develops sensitivity to communicative intentions, the understanding of irony is developed much later.[174-176] At school age the major part of cognitive development, including abstract thinking and skills of conceptualization take place.[174]

2.5.2 Adverse outcome

2.5.2.1 *Fetal growth restriction*

There are several definitions of intrauterine growth restriction, but it is often assumed when the infant is born small for gestational age (SGA), as compared to being appropriate for gestational age (AGA). SGA is sometimes defined as a birth weight less than the 10th or the 5th percentile.[177] In Sweden, the diagnosis of SGA is defined as a birth weight lower than 2 SD below the mean.[178] Additionally, some researchers have defined a birth weight less than 2.5 kilograms as evidence of growth restriction.[24]

Risk factors for fetal growth restriction is use of tobacco, alcohol, narcotics, cocaine, undernutrition, but also fetal infection, chromosomal abnormalities, multiple gestation, pregnancy at high altitude, PE and other maternal diseases such as renal disease, maternal cyanotic heart disease, antiphospholipid syndrome and systemic lupus erythematosus.[72, 102, 177]

Fetal growth restriction can be symmetrical or asymmetrical. An earlier impact, in the first months, often by chromosomal error, teratogens or infection, can give rise to a symmetric restriction, defined by the proportional growth restriction of all organs. It is thought that the number of cell divisions is decreased, thereby influencing all organs equally. On the other hand, a later impact, in the second half of pregnancy, often results in an asymmetric growth restriction. The infant is deprived in oxygen and nutrients and autoregulation redirects the blood flow to the brain. The prioritization of the brain results in a larger head in proportion to the abdomen and a higher brain to liver weight ratio. The underlying mechanism in asymmetric growth restriction is most often some form of placental insufficiency.[24, 71, 177]

One problem with using growth restriction and SGA synonymously is that some infants are constitutionally small and not growth restricted. Furthermore some infants that are constitutionally large will not be regarded as growth restricted even though they are. To overcome this issue, usage of customized growth curves has been proposed.[177] Those growth curves take maternal ethnicity, maternal weight and height, infant sex and parity into account. It is valuable to exclude the constitutionally small and to include constitutionally larger, but growth restricted infants, as outcome is related to growth restriction and not to smallness.[177]

Abnormal Doppler examination of the umbilical artery is related to growth restriction and can be useful in distinguishing pathological growth restriction to constitutional smallness.[125, 177] Also, measurements of blood flow in the fetal middle cerebral artery, the uterine artery, ductus venosus and the umbilical vein have been investigated in relation to growth restriction.[125, 127, 129]

2.5.2.2 *Fetal and neonatal death*

2.5.2.2.1 Intrauterine fetal death (IUFD)

Stillbirth is defined as no signs of fetal life present at or directly after birth, after completed gestational week 22. Causes of IUFD can be divided into fetal, placental and maternal, which often interact with each other. Some causes or underlying etiologies are chorioamnionitis, malformations, growth restriction, placental abruption, hypertensive disorders and DM. In a quarter of the cases no cause can be identified.[71]

The stillbirth rate is the number of stillborn infants per 1 000 live born and stillborn infants.[72] In Sweden, the stillbirth ratio was 40 / 1 000 during the 19th century and since the 1960'ies it has drastically decreased from 20 to around 5 per 1 000 at the new millennium. Many Sub-Saharan and South-Asian countries still have stillbirth rate around 30-40 per 1 000. However, despite obvious advancements in obstetric care, the rate of stillbirth in countries such as Sweden has remained essentially constant during the last 2-3 decades.[7]

2.5.2.2.2 Neonatal death

The neonatal period is defined as the first 28 days of life and hence neonatal mortality is the term that describes a newborn's death within that period. Early neonatal death happens during the first week, while those occurring during week two to four are considered to be late. Perinatal death includes both stillborn infants and neonatal deaths. The neonatal mortality rate is the number of neonatal deaths per 1 000 live born infants, whereas the perinatal mortality rate is the number of stillborn infants and neonatal deaths per 1 000 live- and stillborn infants.[72]

2.5.2.2.3 Infant mortality

Infant mortality is the decease of a live born infant during the first year of life. The infant mortality rate is the number of live born infants who dies during the first year of life per 1 000 live born infants.[72]

2.5.2.3 *Neonatal morbidities*

2.5.2.3.1 Bronchopulmonary dysplasia (BPD)

In the last trimester the fetal lungs prepare for the extrauterine environment, by producing surfactant which prevents the lungs from collapsing during expiration. The risks of developing BPD, oxygen toxicity disease and pulmonary hypertension are increased in premature infants.[72] Depending on how much extra oxygen the infant is in need of and for how long, BPD is regarded as mild, moderate or severe.[179]

2.5.2.3.2 Retinopathy of prematurity (ROP)

As the retinal vessels develop they are very sensitive to excessive oxygen, which often must be administered because of lung immaturity. As a consequence of hyperoxemia, the infant might develop ROP, which can result in blindness.[71]

2.5.2.3.3 Intraventricular hemorrhage (IVH) and periventricular leukomalacia (PVL)

The germinal matrix capillary network in the brain is fragile in the preterm infant; the autoregulation is not fully developed, the matrix does not sufficiently support the vessels and the vessels easily burst in response to an increase in intravascular pressure[72]. As a consequence, preterm infants born before gestational week 32 are at increased risk for periventricular hemorrhage and IVH. The cause of IVH in preterm infants is however multifactorial and prognosis depends on extent of bleeding and location.[72] Some of those hemorrhages resolve, but large lesions can result in PVL, which has been associated to CP.[72, 180, 181] Of preterm infants weighting less than 1.5 kilograms, 3.2% develop PVL.[182] In diagnosing PVL, the presence of cysts is very informative regarding prognosis.[183]

IVH is graded 1-4, where grade 1 is a subependymal hemorrhage, grade 2 an IVH without ventricular dilatation, grade 3 an IVH with ventricular dilatation and 4 an IVH with parenchymal hemorrhage. [184] Long term prognosis is adverse in infants with grade 3 and 4.[182]

2.5.2.3.4 Necrotizing enterocolitis (NEC)

Another complication of prematurity is NEC, primarily affecting low-weight infants[72]. It is thought to be a consequence of bowel ischemia, prematurity and infection. Symptoms include bloody stool, bowel distension, ileus, and can progress to disseminated intravascular coagulation.[72, 182] Out of preterm infants weighting less than 2 kilograms, 3-4% develop NEC.[182]

2.5.2.4 Cerebral palsy (CP)

The definition of CP has changed during the years, and today CP is defined as a group of disorders affecting the development of posture and movement attributed to a non-progressive lesion in early fetal or infant life, often accompanied with impairment in other functions.[185] A spastic CP is described according to which part of the body that is affected, e.g. monoplegia, diplegia, triplegia, quadriplegia or hemiplegia. An extrapyramidal lesion is characterized by movement disorders and/or hypotonia.[182]

Risk factors are prematurity, low birth weight, inflammation and genetical factors. It is only in about 10% that a hypoxic-ischemic event around birth can be identified.[186]

2.5.2.5 Delayed development

The etiology of mental retardation includes genetic causes, metabolic disorders, acquired brain injury, environmental causes, infections and maternal use of alcohol and drugs. In addition sociocultural factors play a role.[187, 188] Genetic causes are more common in severe developmental delay, whereas environmental factors more often

cause mild retardation.[188] Overall, prenatal factors are the most common causes of delayed development.[188]

Development is measured related to the age of the child and is in many scoring systems presented as an index, where the child's developmental age is divided by its chronological age. This is thereafter multiplied by 100 and a normal index is hence 100.[182]

Bayley Scales of Infant and Toddler Development, third edition (Bayley-III) [189] is an individually administered developmental assessment for infants between one and 42 months of age. Bayley-III contains five different domains (adaptive behavior, cognition, language, motor and social emotional) and each domain contains two or more sub-domains. Raw scores from each sub-domain are transformed to scaled scores in order to compare an individual's performance with other children of the same age, and finally the scaled scores are combined into a composite score for each domain.

2.5.2.6 *Adult consequences of fetal life environment*

Life in utero can not only influence the infant in a short term, but has apparently an impact on the future health of the offspring as well.[190] Barker, studying more than 10 000 men in Hertfordshire found that a low birth weight is related to cardiovascular diseases, including stroke, hypertension and DM type II.[191] In rat models fetal undernutrition has been related to hyperphagia in adult life and prenatal hypoxia with atherosclerotic changes in the offspring.[192, 193]

Many have also studied the impact of intrauterine exposure of PE on diseases in adulthood, and a meta-analysis of more than 45 000 patients showed that intrauterine exposure to PE was associated with higher blood pressure and BMI of the offspring in adulthood.[163]

3 AIMS OF THE THESIS

The overall aim of this thesis was to study the placental pathology in relation to maternal symptoms in PE and in relation to neonatal outcome and development.

Specific questions in the studies were:

1. Is there a correlation between placental pathology and the severity of PE?

2. Do patients with severe PE with and without HELLP differ in regard to placental pathology and frequency of SGA?

3. In infants from PE pregnancies, is there a relationship between placental pathology and neonatal outcome?

4. In an extremely premature population, is there a relationship between placental pathology and neonatal outcome?

5. In an extremely premature population, is there a relationship between placental pathology and neurologic outcome and development at 2.5 years of age?

4 MATERIAL AND METHODS

4.1 PLACENTAL EXAMINATION

Pathological variables examined in study I-II were primarily typical PE findings, as the purposes were to correlate placental pathology in PE with maternal symptoms in PE. In study III-IV, more pathology variables were studied in order to include their impact on the outcome of the offspring. This was essential especially in the last study, as its population was not a pure PE population.

The placentas in study II was examined by two different perinatal pathologists: one perinatal pathologist from Karolinska Hospital, Stockholm and one from Free University Hospital, Amsterdam. In addition, they together re-reviewed approximately 20% of the original slides in order to establish common criteria and reach consensus on the histological diagnosis. Macroscopic data was collected from the pathology reports.

In study I, III and IV, all histological slides were reevaluated by one senior perinatal pathologist at Huddinge, who was blinded to outcome. Macroscopic data was collected from the pathology reports.

At the Section of Perinatal Pathology at Karolinska University Hospital Huddinge, macroscopic and microscopic examinations of the placentas were performed as described below.

4.1.1 Macroscopic examination

In nearly all cases the placenta was sent fresh to the Section of Perinatal Pathology at Karolinska University Hospital Huddinge. Upon arrival, the first macroscopic examination was performed, regularly within 48 hours. Thickness and maximal and minimal diameter were measured and the placenta was inspected regarding shape, fetal and maternal surface, membranes and cord. Any abnormality was recorded, in some cases photographed and fresh frozen tissue was obtained in special cases. Thereafter the placenta was fixated as whole in formalin for approximately 8-10 days. During the second macroscopic examination, the placenta was trimmed (membranes and the umbilical cord were removed) and weighed. The placental weight was regarded as normal, low or high in relation to gestational age using a standard curve.[194] The placentas were then sliced in 0.5-1.0 cm thick slices and eventual focal abnormalities were assessed regarding location, color and volume. This is the origin of the data on *amount of infarction*.

Samples were taken for histological analysis from macroscopically normal placenta (2-3), umbilical cord (1-2) and the membranes (1-2) as well as from any focal abnormality observed.

4.1.2 Microscopic examination

Tissue samples from placenta, umbilical cord and membranes were embedded in paraffin and stained with Hematoxylin & Eosin according to standard procedures. The microscopic examination of those slides was repeated in study I, III and IV in a blinded fashion.

Decidual arteriopathy was defined as fibrinoid necrosis of the spiral artery wall, often with dilation of the vessels, with or without the presence of acute atherosis (presence of foamy macrophages) or luminal thrombosis.

Infarction was recognized as an area of ischemic necrosis of the villi. Fresh as well as older infarctions were recorded. Necrotic villi close to intervillous thrombosis and in areas of extensive intervillous fibrin deposition were not regarded as infarction.

The presence of *intervillous thrombosis* was noted, similarly to the infarction. Fetal thrombosis was defined as thrombosis in the vessels in the umbilical cord, chorionic plate or in the stem villi.

Histopathological *abruption* was classified as the presence of retroplacental hemorrhage. Additional signs could include intravillous hemorrhages (fresh abruption), hemosiderin pigment deposition or signs of ischemia in the vicinity of the clot (older abruption). In some of the studies we also included clinical abruption.

Villous maturation was regarded as accelerated when more than 50% of the villi examined in microscopy were more mature than normal for that gestational week. Signs included in the evaluation were the size and configuration of the villi, the trophoblast layer as well as the architecture and size of the blood vessels within the villi. Area around infarctions was not evaluated regarding maturation. In the chronologically first study (study II), all cases were evaluated regarded accelerated villous maturation, but in the following studies we decided to only assess preterm placentas, as it would be impossible to decide whether a full term placenta became mature before term or at term.

Chorioamnionitis was diagnosed histologically in the presence of polymorphonuclear leukocytes. We included all grades of inflammation, from leucocyte infiltration in subchorionic plate, to spreading to the chorion and the amnion, as well as microabcess formation and necrosis of the amniotic epithelium.

4.2   STUDY DESIGNS AND POPULATIONS

Figure 9 illustrates the source populations, rather than the study populations, that the studies in this thesis aimed to investigate, and the relations between those populations.
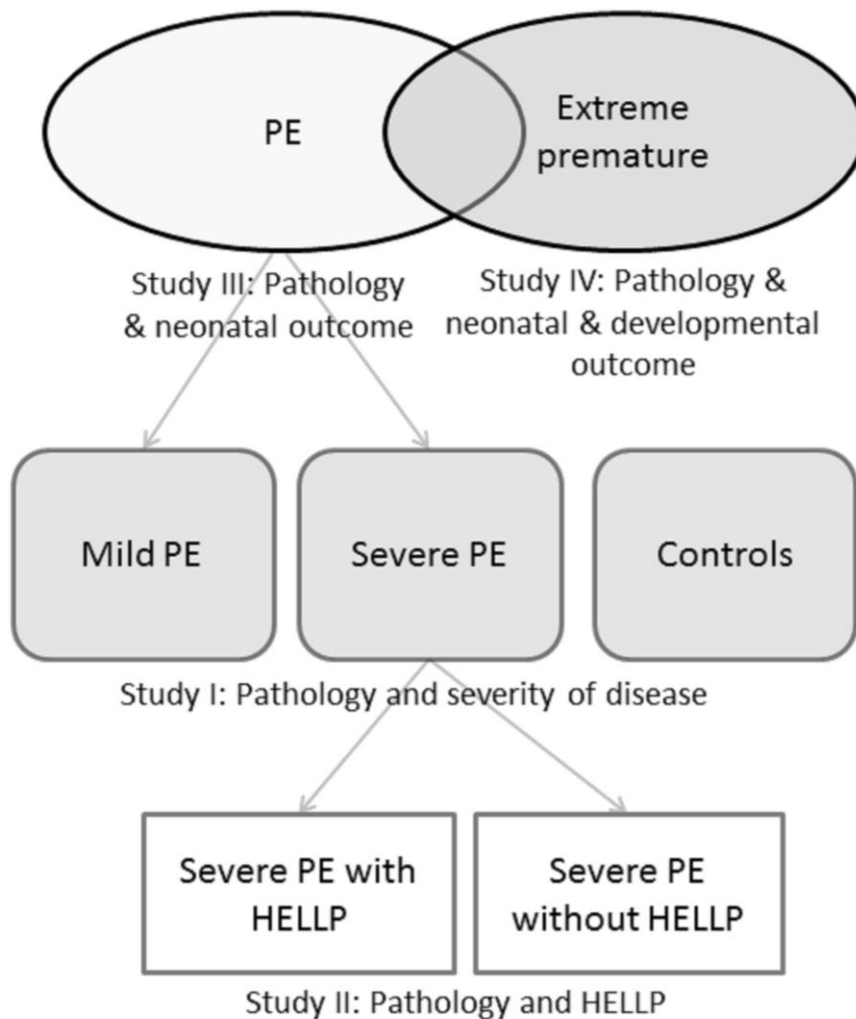
Figure 9. An illustration of the source populations of the studies included in this thesis.

4.2.1   Study I

This study was a case-control study, in which cases were divided into two groups: one with mild PE patients (n=41) and one with severe PE patients (n=116). We also had a control group to compare the two study groups with. Type and extent of placental pathology was compared between the groups.

Cases were recruited from lists of patients with PE cared at Karolinska University Hospital Huddinge and whose placentas were referred for pathological examination at any pathology department in Stockholm. The mild and severe PE-diagnoses were verified by analyzing medical records and laboratory results from all patients.

4.2.1.1 Definition of the PE groups

PE was defined as a blood pressure of 140 mmHg systolic and/or 90 mmHg diastolic or higher on two occasions at least six hours apart and proteinuria, defined as, or analogous to, an excretion of 0.3 grams protein or higher in a 24-hour urine specimen.

Severe PE was diagnosed when the systolic blood pressure reached 160 mmHg, the diastolic 110 mmHg or the proteinuria 5 grams/24-hour sample, but also when the pregnancy was complicated by fetal growth restriction or HELLP syndrome. This is in accordance to the criteria set by the American College of Obstetricians and Gynecologists.[65]

4.2.1.2 The control group

Our inclusion criterion for the patients in the control group was solely that the pregnancy should not be complicated by hypertension. They were selected from a list of placentas sent to the Division of Pathology department between 2004 and 2006 and picked consecutively if fulfilling the criteria. They were matched according to 4 different gestational age groups.

4.2.2 Study II

This was a cohort study consisting of 178 severe PE patients, who were divided into two cohorts: one with HELLP syndrome (n=82) and another without HELLP (n=96). Those were compared regarding type of placental pathology. Also frequency of SGA was compared.

Cases were recruited from lists of patients with PE cared at Karolinska University Hospital Huddinge and whose placentas were referred for pathological examination at any pathology department in Stockholm. The PE- and HELLP diagnoses were verified by analyzing medical records and laboratory results of all patients. Also a Dutch material was gathered, as explained further below.

4.2.2.1 Definition of the cohort

Severe PE was defined as in study I. HELLP was defined as hemolysis, elevated liver enzymes and low platelets. Hemolysis was based on low serum haptoglobin levels < 0.24gram/L and/or elevated lactic dehydrogenase levels > 600U/L. Elevated liver enzymes were defined as ALAT >70 U/l and/or ASAT>70 U/l. Low platelet count was regarded as ≤ 100.000 /μl.

4.2.2.2 Swedish and Dutch material

As HELLP syndrome is a rare disorder, we included HELLP-patients from Free University Medical Center in Amsterdam, The Netherlands, which was a referral hospital for HELLP patients in The Netherlands.

In order to ascertain that the pathological examination at Free University Medical Center and Karolinska University Hospital Huddinge were the same, one senior pathologist from each hospital examined and diagnosed placentas together.

There was a difference in the method of placental weighing of the Swedish and Dutch material. In Free University Medical Center the pathologist weighed the placentas fresh, whereas the placentas at Karolinska University Hospital Huddinge were weighed after formalin fixation. As the placental weight changes after formalin fixation[195, 196] and the Dutch placentas were part of one of the cohorts, there was a risk for confounding. Therefore, only the Swedish placentas were included when placental weight was compared.

Birth weights were related to national growth curves. There were no differences in SGA between the Swedish and Dutch HELLP-patients, when the weights were related to the appropriate growth curve. Of the Swedish HELLP-cases, 6/21 (28.6%) were SGA and of the Dutch 13/60 (21.7%). However, actual birth weight was lower in the Dutch material.

Before we mixed the Swedish HELLP-cohort with the Dutch, we compared them regarding several parameters (maternal smoking, frequency of SGA, accelerated villous maturation, decidual arteriopathy, infarction, intervillous thrombosis and abruption). As no statistical significant differences were found, we compared PE patients with the entire HELLP cohort. One exception was analysis of placental weight, for which the Dutch material was excluded, as explained above.

4.2.2.3   *How many had only HELLP-symptoms?*

Of the HELLP patients, 24 (29.3%) had proteinuria fulfilling the criteria for severe PE. However, measurement of proteinuria was in many cases not done. Regarding blood pressure, we have not recorded whether the pressure reached mild or severe level and we can therefore not report on that. Previously, hypertension or proteinuria has been reported to be absent in up to 15% of HELLP patients.[4]

4.2.3   Study III

This was a cohort study consisting of 544 infants born to PE mothers. The type and extent of placental pathology was correlated to neonatal outcome of the cohort.

Patients were identified from the hospital's diagnostic database and included if the placenta had been referred to the Section of Perinatal Pathology at Karolinska University Hospital Huddinge.

4.2.3.1   *Ultrasound measurements of umbilical artery blood flow*

Often, additional ultrasounds are performed in PE patients. When those were available, we collected data on flood flow of the umbilical artery. An abnormal umbilical artery blood flow was defined as AEDF or REDF, or as a pulsatility index (PI) more than 2 SD above mean at that gestational week.[119]

4.2.3.2   *Neonatal morbidity and mortality*

We choose to study the variable major neonatal morbidity, as many of the severe neonatal morbidities are rare and difficult to study in smaller materials. Major neonatal

morbidity was defined as IVH ≥grade 3, ROP ≥grade 3, NEC, cystic PVL and/or severe bronchopulmonary dysplasia. Additional outcome variables were IUFD, neonatal death, SGA, need of care at neonatal unit, delivery room assessment scores, need of mechanical ventilation and sepsis.

Data on neonatal outcome was gathered from several sources: In 294 of the live-born infants, data could be found in PNQ (Perinatal Quality Registry), while data regarding 227 was gathered from medical records. If data in PNQ was not complete, data was also collected from medical records. For example, if it was recorded that the infant suffered of BPD, but the % of Oxygen at 36 weeks was not specified, we went through the medical records to determine if the BPD was severe. Also, cases with sepsis were verified by analyzing the medical records. Data on ROP was gathered from the National Registry of ROP.

4.2.4 Study IV

This cohort study was different from the previous studies because it did not include a pure PE population, but an extremely premature population, comprising both PE and non-PE patients. The patients were part of the extremely preterm infants in Sweden study (EXPRESS). The placental pathology was related to both neonatal outcome as well as neurologic outcome and development at 2.5 years of age.

The patients included were all live-born and stillborn infants born in gestational week 22+0 - 26+6 in the Stockholm County during April 2004 – March 2007, where a placental examination was performed at any hospital. A placental examination was done in 79% of the patients. After excluding two placentas because of poor quality of the sample, 167 patients remained.

4.2.4.1 Neonatal morbidity and mortality

Variables studied in the neonatal period were almost the same as in study III. However, there was no interest in studying admission to neonatal care as all infants born <27 weeks of gestational age are in need of neonatal hospital care. Data was collected from PNQ and if something was unclear or missing, medical records were analyzed.

4.2.4.2 Neurologic outcome and development at 2.5 years of age

Neurologic impairment was divided into severe neuromotor impairment, defined as CP, and severe neurosensory impairment, defined as severe hearing impairment (hearing loss that could not be corrected despite a hearing aid) and/or severe visual impairment (blind or only able to see torch light).

At 2.5 years corrected age a Bayley-III tests were performed to evaluate developmental outcome.[189] We have used a group of healthy term born Swedish children as a reference.[197] A score more than 2 SD below the mean of their results was considered delayed, i.e. a composite cognitive score below 83, a composite language score below 85, and a composite motor score below 80.

4.3 STATISTICS

For demographic data we performed t-tests when analyzing continuous data that was normally distributed. If the data was not normally distributed we used the non-parametric test Mann-Whiney U-test. For categorical data we performed Chi-square test and if >20% of expected frequencies were <5 or if any expected frequency was <1, we used Fisher's exact test.

4.3.1 Study I

For analysis on binomial and multinomial outcome variables we used logistic regression analysis. Adjustment for gestational age was done when it was shown to affect the result. For placental weight in relation to gestational age we used chi-square test. In the regression analyses we first analyzed the effect on all groups and if it was significant we continued analyzing differences between the three groups. In order to compare three groups (mild PE, severe PE and controls) in the regression model, we used Dummy-variables.

4.3.2 Study II

When analyzing binomial and multinomial outcome variables we used logistic regression and when gestational age was shown to affect the result, gestational week was included in the analysis as a covariate.

4.3.3 Study III

For outcome variables, forward step-wise analyses were performed including all pathological variables and the gestational week. Including all variables in one analysis model was possible because of the larger number of patients compared to study I and II. Also, interactions were studied and further analyzed. When analyzing the more rare neonatal morbidities separately, we used Fisher's exact test.

4.3.4 Study IV

For outcome variables we used forward stepwise analysis including variables that were significant in the univariate analysis. We did not include all pathological variables in the model analysis in order to keep events per variable preferably below 10.[198] In addition to pathological variables also gestational age, presence of PE, obesity and maternal age were studied and included in the models if they showed a significant effect on the outcome. Also interactions were studied and included in the analyses when present.

For analysis of the relations between pathology and neurologic and developmental outcome we used Fisher's exact tests for categorical data as <20% of expected frequencies were <5 or any expected frequency <1. Regarding continuous development data, we performed a regression analysis (ANCOVA) including gestational age in the analysis. The data on placental weight did not fulfill the criteria for a regression analysis and was performed with a non-parametric test.

4.4 ETHICS PERMISSION

For the first three studies, ethic approvals to perform the studies without contacting the patients were given from the Regional Research Ethics Board in Stockholm, Sweden. All patients that had their placenta sent to the division of pathology gave their informed consent to save the placental tissues according to the bio bank law. Tissues from patients who disapprove are destroyed after examination and histopathology slides are not stored in those instances. According to Dutch law, no ethics permission was needed for the Dutch material included in study II.

For study IV, ethics approval was given from the Regional Ethics Review Board in Lund, Sweden. The reason why the Board in Lund was engaged was that the study was part of a nationwide study that the Review Board in Lund gave its approval to. The parents gave their written consent for data acquisition and for examination of their children.

5 RESULTS

5.1 STUDY I

The 157 PE patients were diagnosed with PE at mean gestational week 33 and delivered at mean gestational week 35. The gestational weeks at diagnosis and delivery were later in the 41 mild PE patients (diagnosis in week 36 and delivery in week 37) than in the 116 severe PE patients (diagnosis in week 33 and delivery in week 34) (p<0.001).

The non-PE group (controls) consisted of patients without any hypertension in their pregnancies and they were matched to the PE-patients according to gestational age group. Many of them were premature and admitted for placental examination for other pathological reasons than hypertension in pregnancy. Infection was found in 27% and abruption in 23% of the patients in the non-PE-group. Also, growth restriction (17%) and stillbirth or neonatal deaths (12%) were relatively common reasons for referral. Whereas stillbirth was more common in the non-PE group (11.5%) than in the PE group (3.2%) (p<0.01), SGA-infants were less common in the non-PE-group (29.3%) than in the PE-group (47.8%) (p<0.001).

5.1.1 Placental pathology

There were significant differences between mild and severe PE patients in relation to the non-PE group regarding infarction (p<0.001), decidual arteriopathy (p<0.001) and accelerated villous maturation (p<0.001), as is illustrated in figure 10. No differences were found regarding intervillous thrombosis or abruption. When comparing mild and severe PE patients a difference could only be found in the occurrence of infarction.
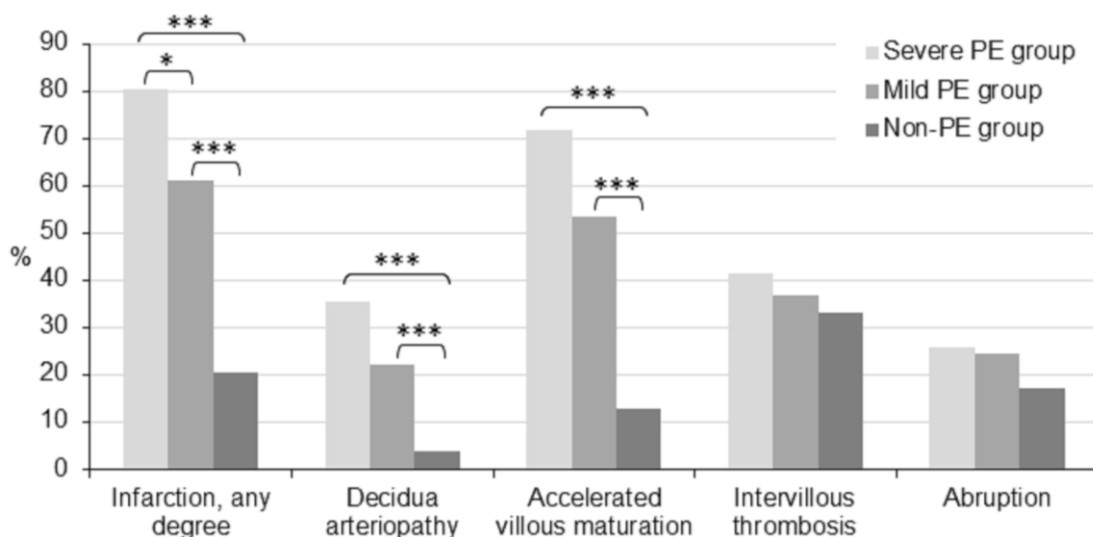
Figure 10. Placental pathology in the severe PE-, mild PE- and non-PE-group (controls) in study I.

When studying the degree of infarction we found significant differences between the non-PE group and the two PE-groups (p<0.001), but also between the mild and severe PE groups (p=0.015). Se figure 11.

38
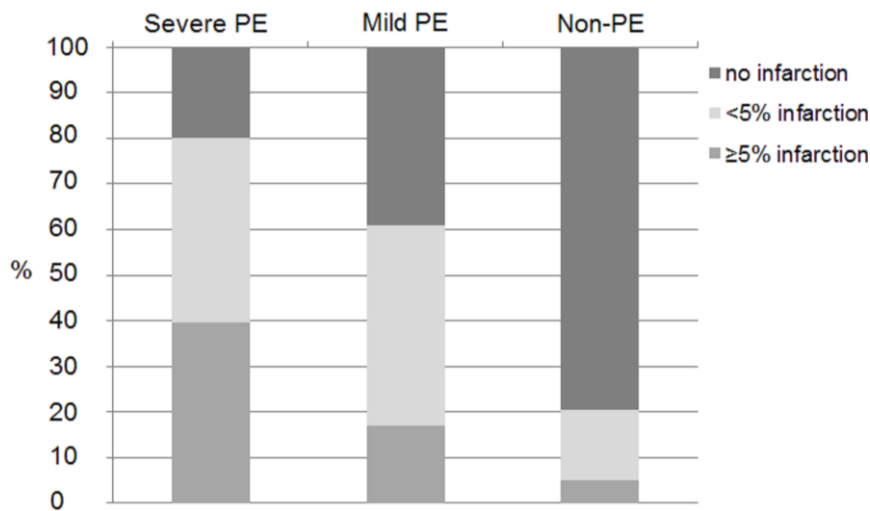
Figure 11. Amount of infarction in the severe PE-, mild PE- and non-PE-groups (controls) in study I.

Regarding placental weight, there were no significant differences in the weights in relation to gestational age between the mild PE patients, the severe PE patients or the non-PE patients.

5.1.2 Sub groups

According to current diagnostic criteria, PE is regarded as severe when the mother has severe hypertension, severe proteinuria, HELLP syndrome or when the infant is growth restricted. In the severe PE group, 43 cases had both severe maternal symptoms and a SGA-infant. In 42 women, the criteria for severe PE were fulfilled without a SGA-infant. In 31 cases, the babies were SGA although the maternal symptoms were at a mild PE level. According to the above criteria, all those were severe PE. Some aspects of placental pathology in the sub groups of severe PE and in mild PE and non-PE are presented in figure 12.
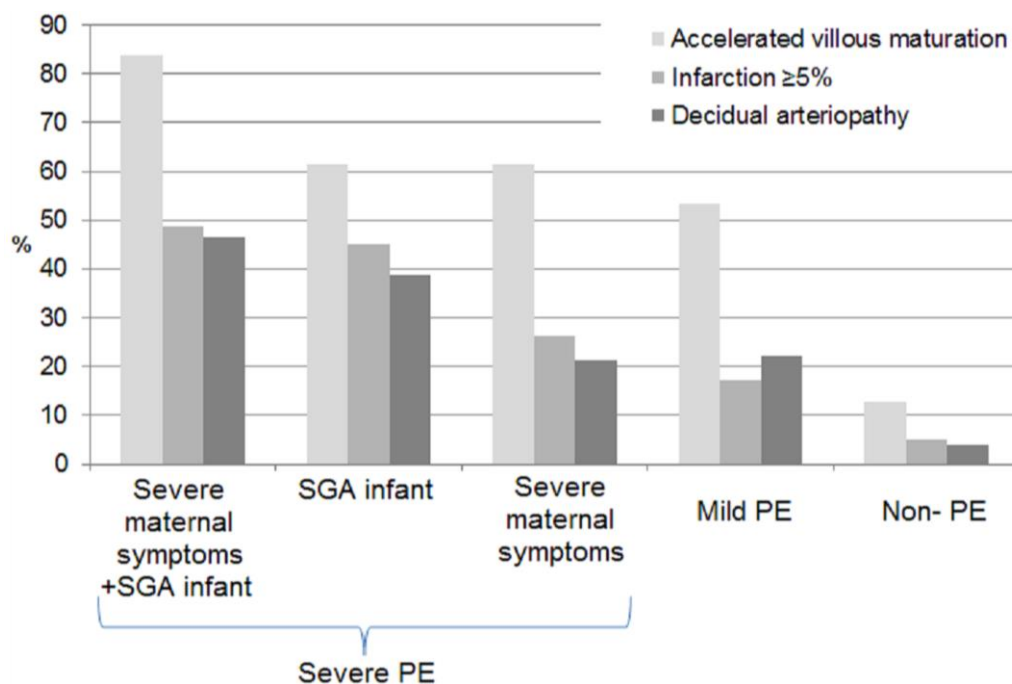
Figure 12. Placental pathology in sub groups of severe PE, mild PE and non-PE-patients in study I.

5.2 STUDY II

Patient demographics did not differ significantly between the 117 PE patients without HELLP syndrome (PE-group) and the 82 severe PE patients with HELLP syndrome (HELLP-group), with the exception of gestational week. Mean gestational week at delivery was 34 in the PE group and 32 in the HELLP-group (p=0.004). The time between diagnosis and delivery was slightly shorter (about half a week) in the HELLP-group (p=0.06).

5.2.1 Placental pathology

In the PE-group, there were significantly higher frequencies of infarction (p=0.014), intervillous thrombosis (p<0.001) and abruption (p=0.002) compared to the HELLP-group, see figure 13. There were no significant differences in accelerated villous maturation or decidual arteriopathy.

In this study, originally all cases were included in the analysis of accelerated villous maturation. When excluding term placentas (14 PE-cases and 12 HELLP-cases), accelerated villous maturation was present in 72.1% of the PE-patients and in 81.4% of the HELLP-patients, as is seen in figure 13. Excluding term cases did not change the result (data not shown).
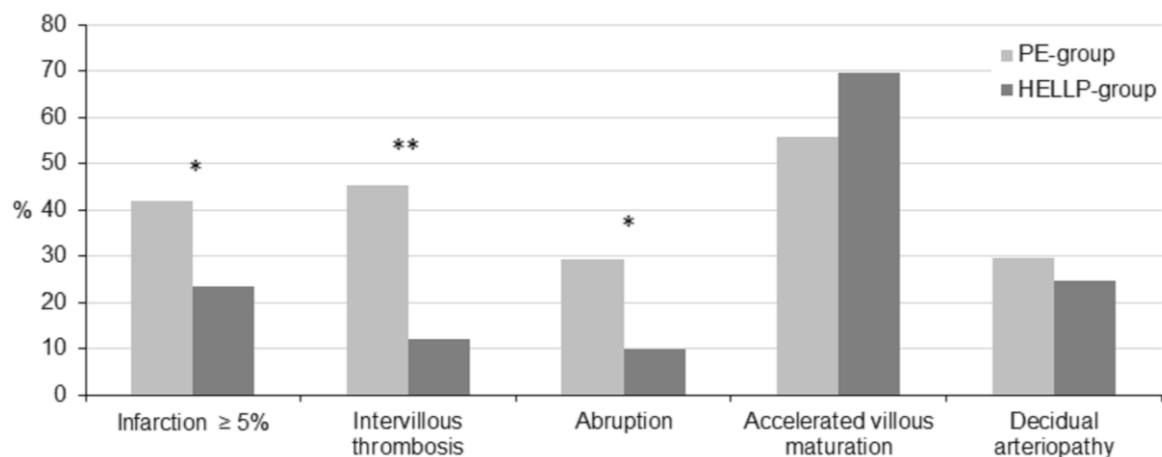
Figure 13. Placental pathology in PE- and HELLP-patients in study II.

5.2.2 Placental weight

The mean placental weight in the PE-group was 310.5 gram and in the HELLP-group 391.6 gram (p<0.001). Regression lines for placental weight in the two groups according to gestational age are presented in figure 14. When studying the placental weight in relation to gestational age according to a standard curve, there were significantly more high placental weights in the HELLP-group (p=0.016).

One placenta had an extreme weight of 1008 gram, and was also reported to be hydrops fetalis. That case was excluded when calculating the mean placental weight as well as in the regression analysis.
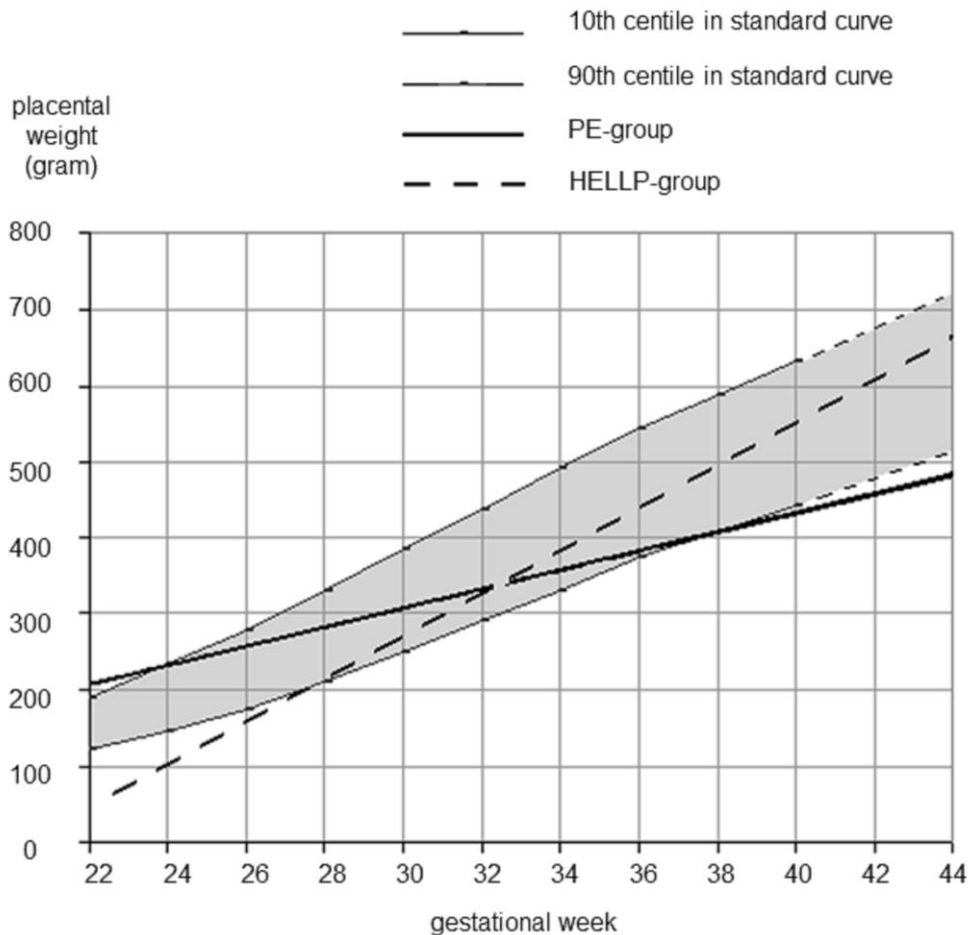
Figure 14. Regression lines for placental weight according to gestational age in PE- and HELLP-patients in study II.

5.2.3 Birth weight

There was a significant difference (p<0.001) in frequencies of SGA-infants between the groups. In the PE-group, 71.9% were SGA and in the HELLP-group 23.5%. Mean birth weight, adjusted for gestational age was 1562.7 grams in the PE-group and 1672.1 grams in the HELLP-group. The difference in birth weight was dependent on gestational age. In gestational week 30, the difference was not significant, but in week 33 it was (p=0.03). In week 36, the difference was even larger (p<0.001).

5.3 STUDY III

Out of the 544 PE patients, 172 women gave birth very preterm (before gestational week 32), 207 women delivered preterm (between gestational week 32 and 37) and 165 women at term (from gestational week 37).

5.3.1 Placental pathology

Placental pathology of the entire population is presented in figure 15. The most common findings were accelerated villous maturation (evaluated in preterm cases) (73.1%), intervillous thrombosis (32.7%), decidual arteriopathy (28.7%) and infarctions involving ≥5% of the placenta (17.1%).
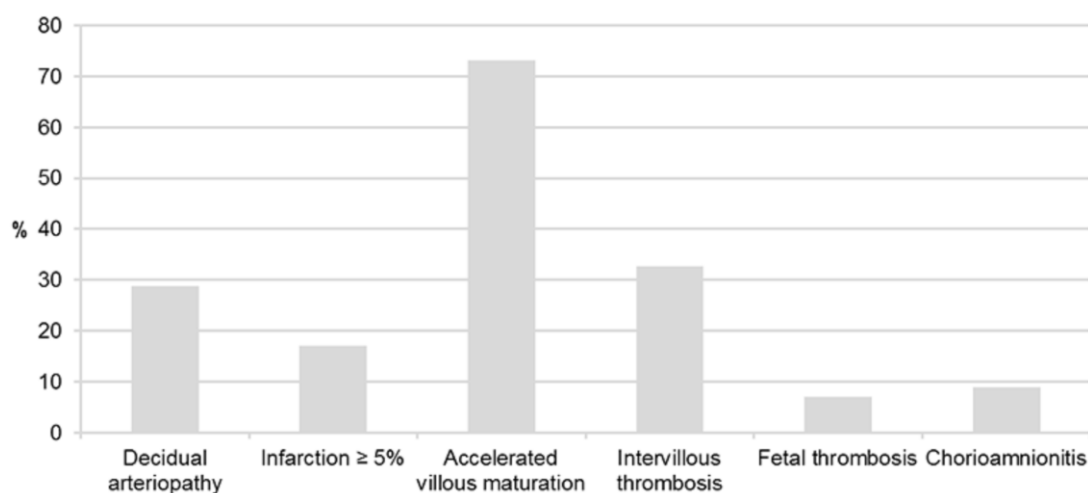
Figure 15. Placental pathology in the study cohort in study III.

5.3.2 Umbilical artery blood flow

Measurement of the umbilical artery blood flow was performed in 317 women. It was abnormal in 113 (35.6%). Regression analysis on associations between placental pathology and blood flow is presented in table 3. An abnormal umbilical artery blood flow was related to ≥5% placental infarction and a low placental weight (adjusted odds ratio (OR) 4.7, 95% confidence interval (CI) [1.9-12], p<0.001; adjusted OR 4.3, 95% CI [2.2-8.5], p<0.001; respectively), whereas intervillous thrombosis and accelerated villous maturation were inversely related to abnormal umbilical artery blood flow (adjusted OR 0.46, 95% CI [0.25-0.85], p=0.01; adjusted OR 0.39, 95% CI [0.17-0.91] p=0.03; respectively).

Table 3. Regression analyses of placental pathology & umbilical artery blood flow

	Adjusted OR (95% CI)	Model p-value[a]
Low placental weight	4.3 (2.2-8.5)	<.001
Accelerated villous maturation	0.39 (0.17-0.91)	.04
Decidual arteriopathy	0.76 (0.41-1.4)	*NS*
Infarction		
<5%	1.5 (0.75-3.1)	.002
≥5%	4.7 (1.9-12)	
Intervillous thrombosis	0.46 (0.25-0.85)	.01
Fetal thrombosis	1.4 (0.51-4.1)	*NS*
Villitis	1.4 (0.63-3.3)	*NS*
Chorioamnionitis	0.75 (0.20-2.8)	*NS*
Gestational age, increase/week	0.71 (0.64-0.80)	<.001

OR, odds ratio; *CI,* confidence interval., [a]Forward-stepwise model.

5.3.3 Perinatal and neonatal outcome

Fetal mortality was 4.2% (n=23) whereas neonatal mortality was 1.7% (n=9). The frequency of SGA-infants was 39.8% (n=216). Delivery room assessment showed a 5 minutes Apgar score <4 in 1.3% (n=7) and <7 in 7.9% (n=41) of the live born infants.

Base excess (BE) of the umbilical artery was ≤-10 in 14.4% (n=41) of the infants and pH of the umbilical artery ≤7.10 in 6.9% (n=27). Data on BE and pH was not available in all infants.

Admission to neonatal care differed depending on gestational age: all (n=151) infants born very preterm and the majority of the preterm infants (n=184, 89.3%) were admitted, in contrast to a minority (n=30, 18.3%) of the term infants.

Major neonatal morbidity was found in 23 (15.2%) of the very preterm infants. Three of them had IVH ≥grade 3, nine had ROP ≥grade 3, ten had NEC, two had cystic PVL and four had severe BPD. Five of the infants had two major morbidities, but none had more than two. Of the very preterm infants 59 (39.1%) needed mechanical ventilation and 50 (33.1%) suffered from sepsis.

5.3.4 Placental pathology and outcome

Logistic regression analyses on associations between placental pathology and some neonatal outcome are shown in table 4. Whereas infarction and chorioamnionitis were associated with a higher incidence of IUFD, accelerated villous maturation was associated with a lower one. SGA was associated with a low placental weight and infarction. Admission for neonatal care was associated with decidual arteriopathy and fetal thrombosis. For OR and p-values see table 4. No associations were found with need for mechanical ventilation or sepsis.

Regarding delivery room assessment, chorioamnionitis was associated with Apgar score <7 at 5 minutes (adjusted OR 5.2, 95% CI [1.6-18]) and a low umbilical artery pH (adjusted OR 3.3, 95% CI [1.0-11]). Also infarctions ≥5% was associated with BE ≤-10 (adjusted OR 5.4, 95% CI [1.8-16]).

Major neonatal morbidity, which was evaluated in the very preterm infants, was associated with a low placental weight in relation to gestational age (adjusted OR 5.2, 95% CI [1.1-24]). Also a high placental weight was associated with major neonatal morbidity (adjusted OR 1048, 95% CI (21-51663). None of the women with a large placenta had DM. The distribution of placental weight in relation to gestational week according to a reference curve in infants with and without major neonatal morbidity is presented in figure 16. No preterm large placenta (n=12) showed accelerated villous maturation, compared to 75.8% of preterm placentas with normal or small size (n=326) (Fisher exact test: p<0.001).

When analyzing the major neonatal morbidities separately using Fishers exact test, accelerated villous maturation tended to be inversely related to IVH ≥ grade 3 (p=0.07). A low placental weight was associated with NEC (p<0.05), whereas a high weight tended to be related to cystic PVL and IVH ≥ grade 3 (p=0.05 and p=0.08, respectively). Decidual arteriopathy was associated with ROP ≥ grade 3 (<0.05), while infarction tended to be inversely related with ROP ≥ grade 3 (p=0.06).

Table 4. Placental pathology and perinatal outcome

	IUFD		SGA		Admission to neonatal unit	
	Adjusted OR (95% CI)	Model p-value[a]	Adjusted OR (95% CI)	Model p-value[a]	Adjusted OR (95% CI)	Model p-value[a]
Low placental weight	3.9 (0.71-21)	NS	7.2 (4.5-11)	<.001	0.97 (0.49-1.9)	NS
Accelerated villous maturation	0.18 (0.04-0.77)	.04	0.82 (0.45-1.5)	NS	5.1 (0.50-51)	<.001[b]
Decidual arteriopathy	1.9 (0.6-6.4)	NS	1.3 (0.79-2.0)	NS	2.7 (1.1-6.5)	.04
Infarction						
<5%	11 (0.95-134)	<.001	1.4 (0.85-2.2)	<.001	0.83 (0.39-1.8)	NS
≥5%	75 (5.5-1011)		3.2 (1.7-5.9)		1.7 (0.63-4.8)	
Intervillous thrombosis	1.1 (0.31-4.0)	NS	1.3 (0.87-2.1)	NS	1.9 (0.96-4.0)	NS
Fetal thrombosis	0.37 (0.02-6.7)	NS	1.2 (0.56-2.7)	NS	2.4 (0.84-6.7)	.02
Villitis	3.1 (0.47-21)	NS	1.6 (0.90-3.0)	NS	1.8 (0.79-4.2)	NS
Chorioamnionitis	14 (1.6-125)	.03	1.3 (0.60-3.0)	NS	2.2 (0.78-6.0)	NS
Gestational age, increase/week	0.51 (0.39-0.67)	<.001	0.91 (0.84-0.98)	<.001	0.59 (0.45-0.78)	<.001

IUFD, intrauterine fetal death; *SGA,* small for gestational age; *OR,* odds ratio; *CI,* confidence interval.

[a]Forward-stepwise model; [b]Accelerated villous maturation remained in the stepwise analysis because of the large number of full term placentas. When excluding those, there was no association (adjusted OR 5.1 [0.5-50.9], p=.18).
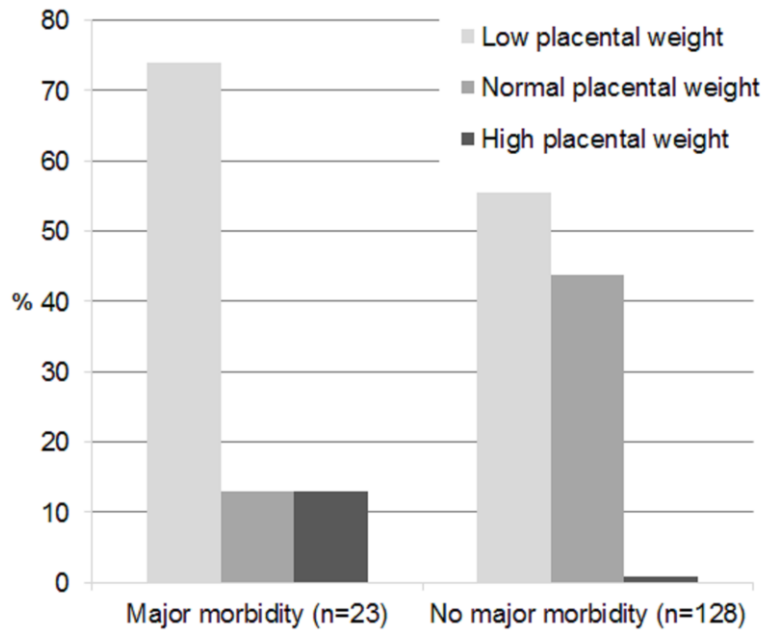
Figure 16. Placental weight in relation to gestational age in infants with or without major neonatal morbidities in study III.

5.4 STUDY IV

Out of the 167 women giving birth to extremely premature infants, 13 were diagnosed with PE. Of the women with available weight and height, 54 (43.2%) were overweight and 22 (17.6%) were obese. Cesarean section was performed in 36 (21.6%) of the women.

5.4.1 Placental pathology

Placental pathology of the entire population is presented in figure 17. The most common findings were chorioamnionitis (61.7%), accelerated villous maturation (evaluated in preterm cases) (26.9%) and fetal thrombosis (19.8%). Of 13 women diagnosed with PE, 84.6% had accelerated villous maturation compared with 21.1% of the 154 without a PE diagnosis (p<0.001, Fishers exact test).
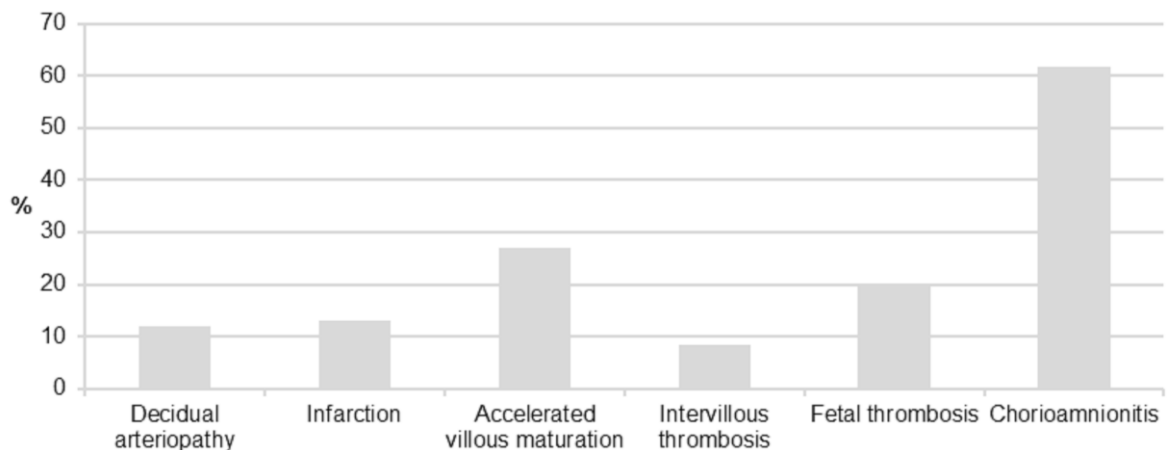
Figure 17. Placental pathology in the study cohort in study IV.

5.4.2 Outcome of the offspring

5.4.2.1 Perinatal and neonatal outcome

Fetal mortality was 37.1% (n=62) and neonatal mortality 36.2% (n=38). Birth weight was SGA in 29 (18.5%) of the infants. At 5 minutes, 47 (45.2%) had an Apgar score <7. Major neonatal morbidity was found in 42 (40.0%) of the neonates.

5.4.2.2 Neurologic outcome and development at 2.5 years of age

Information on CP was available in 58 children, and of them three were diagnosed with CP. None had severe visual or hearing impairment.

At 2.5 years corrected age, 50 were examined regarding cognitive (n=50), language (n=49) and motor (n=48) development. Mean Bayley-III cognitive score was 96.00 (SD 8.3) and of these one (2.0%) fulfilled the criteria for a delayed cognitive development. Looking at language development, mean Bayley-III language score was 96.51 (SD 14.4). Seven (14.3%) of them had a delayed language development. When studying motor development, mean Bayley-III motor score was 101.29 (SD 17.2) and five (10.4%) of them had a delayed motor development.

5.4.3 Placental pathology and outcome of the offspring

5.4.3.1 Placental pathology and neonatal outcome

Fetal thrombosis and low placental weight were associated with both IUFD and SGA infants. Chorioamnionitis was associated with a lower frequency of SGA. Accelerated villous maturation was associated with better Apgar score at 5 minutes. For OR and p-values se tables 5-7. Major neonatal morbidity and neonatal mortality was not significantly associated with any placental pathology variable.

Table 5. Regression analyses of placental pathology and Apgar <7 at 5 minutes

	Univariate analysis		Model analysis	
	p-value	Crude OR (95% CI)	p-value*	Adjusted OR (95% CI)
Abruption	.92	0.96 (0.43-2.1)	-	-
Accelerated villous maturation	.0046	0.23 (0.085-0.64)	.041	0.33 (0.12-0.96)
Chorioamnionitis	.32	1.5 (0.66-3.5)	-	-
Decidual arteriopathy	-	-	-	-
Fetal thrombosis	.45	0.63 (0.20-2.0)	-	-
Infarction	.11	0.27 (0.055-1.4)	-	-
Intervillous thrombosis	.81	1.2 (0.24-6.4)	-	-
Low placental weight	.72	0.82 (0.29-2.4)	-	-
Obstetrical data:			-	
Gestational week	<.001	0.52 (0.37-0.73)	<.001	0.56 (0.40-0.79)
Obesity	.85	0.90 (0.30-2.7)	-	-
Maternal age	.82	0.99 (0.93-1.1)	-	-
PE	.25	0.38 (0.07-2.0)	-	-

* Forward-stepwise model

Table 6. Regression analyses of placental pathology and IUFD

	Univariate analysis		Model analysis	
	p-value	Crude OR (95% CI)	p-value*§	Adjusted OR (95% CI)
Abruption	.049	0.49 (0.24-1.0)	.089	0.61 (0.27-1.4)
Accelerated villous maturation	.92	1.0 (0.51-2.1)	-	-
Chorioamnionitis	.041	0.51 (0.27-1.0)	.26	-
Decidual arteriopathy	.0087	3.7 (1.4-9.9)	.16	-
Fetal thrombosis	.0080	2.9 (1.3-6.3)	<.001	5.3 (2.0-13.8)
Infarction	.075	2.3 (0.92-5.6)	.34	-
Intervillous thrombosis	.11	2.4 (0.81-7.4)	-	
Low placental weight	.0068	2.8 (1.3-5.9)	.011	4.2 (1.7-10.3)
Obstetrical data:				
Gestational week	<.001	0.66 (0.52-0.83)	<.0001	0.53 (0.39-0.71)
Obesity	.18	0.48 (0.16-1.4)	-	-
Maternal age	.99	1.0 (0.94-1.1)	-	-
PE	.92	1.1 (0.33-3.4)	-	-

* Forward-stepwise model, § Including interactions

Table 7. Regression analyses of placental pathology and SGA

	Univariate analysis		Model analysis	
	p-value	Crude OR (95% CI)	p-value*	Adjusted OR (95% CI)
Abruption	.023	0.27 (0.09-0.84)	.29	-
Accelerated villous maturation	.053	2.3 (1.0-5.4)	.54	-
Chorioamnionitis	<.001	0.20 (0.08-0.47)	.014	0.25 (0.08-0.74)
Decidual arteriopathy	.011	3.7 (1.3-10.1)	.51	-
Fetal thrombosis	<.001	4.7 (1.9-11.2)	<.001	7.2 (2.2-22.9)
Infarction	.0054	4.0 (1.5-10.5)	.56	-
Intervillous thrombosis	.24	2.1 (0.6-7.4)	-	-
Low placental weight	<.0001	13.7 (5.3-35.1)	<.001	8.7 (2.8-27.6)
Obstetrical data:				
Gestational week	.037	1.4 (1.0-1.8)	.94	-
Obesity	.94	1.0 (0.31-3.5)	-	-
Maternal age	.30	1.0 (0.97-1.1)	-	-
PE	<.0001	14.0 (3.9-49.6)	.033	5.1 (1.1-23.0)

* Forward-stepwise model

5.4.3.2 Placental pathology and neurologic outcome and development at 2.5 years

Firstly, placental infarction showed a significant association with a diagnosis of CP (p=0.036). Furthermore, placental abruption showed a tendency to be associated with CP (p=0.061).

Of the 34 children with normal villous maturation, 7 (20.6%) were classified as being delayed with their language development, whereas none of the 15 children with accelerated maturation had a delayed language development. The difference did not reach the significance level, but showed a tendency (0.084).

Fetal thrombosis showed a tendency to be related to a lower cognitive development (mean 91.7, SD 8.7) compared with children without fetal thrombosis (mean 97.0, SD 8.0). After adjustment for gestational age the difference still did not reach a significance level, but showed a tendency (p=0.055). Including sex in the analysis did not change the result (data not shown).

Lastly, infants with a low placental weight showed a mean Bayley-III composite motor score of 92.0 (SD 15.1), compared with children without a low placental weight whose mean Bayley-III composite motor score were 103.4 (SD 7.1). This tended to be significant (p=0.051), when analyzed with a non-parametric test. A regression analysis was not performed as the data did not fulfill the criteria for such analysis.

6 DISCUSSION

6.1 METHODOLOGICAL CONSIDERATIONS

6.1.1 Study designs

The studies included in this thesis are all observational cohort- or case-control studies. Some of them are retrospective, others prospective. The two first studies are solely retrospective.

One disadvantage with retrospective studies is the dependence on information that is available. It is impossible to go back in time and reexamine the sick patient, study the placenta macroscopically again, take any additional laboratory tests or add any other information that is lacking in the medical record.

In the second study, two different perinatal pathologists examined the placentas histologically and were not blinded to study group. Their evaluations of the placentas might have differed, which could have influenced the results as they examined different proportions of the study groups. An attempt to minimize this problem was done by letting the pathologists examine several cases together. In addition, the pathologists who examined the placentas could have been prone to find certain pathological features depending on study group, as they were not blinded. Further possible misclassifications are discussed under the section misclassification.

In the first study (chronologically the second), we tried to overcome some of the above issues by letting one experienced perinatal pathologist reexamine all histopathological slides of placental tissue, blinded to study group. However, the macroscopic data could not be reexamined.

In the first study, we also included a control group. Recruiting a control group to this sort of study population is complicated. The morphology of the placenta changes with gestational week, thus the inclusion of healthy term placentas as controls is obviously suboptimal. It is therefore desirable to find a healthy control group of the same gestational age. However, recruitment of such an ideal control group is unachievable, since the overwhelming majority of placentas from preterm pregnancies are referred to pathologic examination because of a pregnancy complication. In the choice between two suboptimal control groups, we used placentas from all gestational weeks, not complicated by hypertension, but by other disorders. It is nonetheless important to remember the type of reference group when drawing conclusions from the results. No differences could be found regarding intervillous thrombosis and abruption, which could be explained by the non-ideal control group.

The two last studies were mainly prospective cohort studies. Although some data was gathered retrospectively (depending on which year between 2000 and 2009 the infant was born), the cohorts were identified before knowledge of the outcome. Also, in those studies, one senior perinatal pathologist examined the placental pathology, blinded to

outcome. The outcome data was gathered from medical records and data registries, as well as from the prospective follow-up of children at 2.5 years of age.

One disadvantage of the last two studies is that some of the neonatal morbidities and developmental variables are rare. In order to find associations, the study groups must be very large. To compensate for that, we chose to study more frequent variables too, as well as a commonly used composite variable, including the most severe diseases in the neonatal period. In order to study associations with the rare diseases separately, case-control studies would be more suitable.

6.1.2 Type I and type II errors

Thanks to statistical analyses, we drew conclusions on whether differences or associations were likely to be true or not. However, it is possible to draw the wrong conclusion. Assuming there is an association (p-value <0.05), when it is not true, one makes a type I error.[199] Choosing a significance level at p <0.05 means that there is a 95% probability that the result is true, when the p <0.05. However, there is also a 5% probability that it is not. Performing 100 analyses, 5 of them will show significant associations without actually being true associations. In our studies we have performed many analyses and the risk of type I error is high. In this thesis, there are in total about 35 associations or differences with a significance level at p <0.05. Out of those, 5%, hence 1 or 2, are perhaps type I errors.

On the other hand, to assume that there is no association (p-value >0.05), when there actually is one, represents a type II error.[199] This is common when lacking power, meaning that there are too few cases in order to note a difference or an association that actually is true. This can also have occurred in our studies, as the number of cases included was limited to the placentas referred for pathological examination.

6.1.3 Biases

Biases are systematic errors resulting in wrong conclusions. Making a study larger does not minimize this type of error, but if one identifies possible biases one can design a study to avoid them. Bias is often divided into information bias, confounding and selection bias.[200] Information bias will be dealt with under the title misclassification.

6.1.3.1 Missclassification of placental pathology

The placental feature *decidual arteriopathy* is a focal finding located at the maternal side of the placenta, not all of which is included in the specimen sent for pathologic examination. If not enough decidual arteries are represented in the placental specimen, the pathologist might not detect the arteriopathy although it was present in the maternal vessels. The frequencies of arteriopathy differed between 22-35% in the PE groups we studied. Gerretsen et al[201] showed absent physiological changes of the spiral arteries in 97% of cases in a PE population. Also, Meekins et al[16] found atherosis in the decidual arteries of 92% of their PE patients. They both, compared to us, studied biopsies taken from the placental bed after delivery.

It is therefore likely that this feature is underestimated in our studies. However, as the examination of the placenta follows a structured manual and as most of the studies were blinded, there should not be any difference of this type of error in placentas from different groups. Hence the type of error is what is called non-differential and there is a risk to underestimate the frequency in any group, making it more difficult to find a difference or an association that actually is present.

There is a physiological *maturation of the villi* with gestational age and the exact estimation of maturation of the villi has been shown to be difficult. A difference of up to 6 gestational weeks can remain undetected[202]. A previous study[202] showed that the pathologists tended to overestimate the age of early gestations and underestimate the age of later gestations. Anyhow, this error will be equally common in all groups, again non-differential. An exception is study I in which mild PE, more often late, is compared to severe PE, more often early. Another difficulty regarding maturation is that it is impossible to know whether a term placenta became fully matured at the right time or too early. The term placentas can therefore not be evaluated regarding accelerated villous maturation.

Placental infarctions can be seen grossly, but should be verified microscopically. Otherwise, there is also a risk for misclassifying. Becroft et al[33] reported that almost a quarter of their macroscopical findings were misclassified. The most common misclassification was the macroscopical identification of a possible infarction that microscopically showed to be plaques of intervillous fibrin. The fact that we always diagnosed placental infarction microscopically is a major strength of this thesis.

Formalin fixation is shown to increase the *placental weight* by approximately 10%.[195, 196] The Swedish placentas were weighed after formalin fixation and the Dutch before. As the Dutch material only belonged to one of the study groups, this could have resulted in a differential error, which could have led to the assumption that HELLP patients have placentas with lower weight, a conclusion not necessarily true. As the placentas were weighed differently in Sweden and Holland, we excluded the Dutch material regarding this parameter, thus decreasing the risk of bias.

Fetal thrombosis can be postmortem in IUFD[13] and as there is no technique to distinguish pre- and postmortem thrombosis, it is impossible to know if fetal thrombosis or death came first.

Placental abruption is also a matter worth discussing. Not ideally, we have classified abruption differently in the studies included in this thesis. In the first two studies and in the last study, we included both clinically and pathologically diagnosed abruptions. In the third study we did not include the clinical ones, but neither did we analyze abruption in that study, as we focused on other variables.

Oyelese et al[36] argued that abruption is a clinical diagnosis and that a pathological examination regarding abruption should be avoided unless there was an adverse outcome of the pregnancy. This sounds reasonable, but we were interested in the impact of a histologically diagnosed abruption and its correlation with outcome. Therefore we never excluded the pathological diagnosis. Clinically and pathologically

diagnosed abruptions do differ as a clinical often is an acute, whereas a pathologically diagnosed abruption more often is chronic. In an ongoing parallel study[203], it was shown that histologic abruption was correlated to hypoxic ischemic encephalopathy and adverse neonatal outcome, suggesting that the histopathological signs of abruption in the placenta may be important indicators, independent of the clinical picture.

Inter-observer variability is generally high in placental pathology, especially if no agreement of diagnostic criteria has been settled.[204-206] By letting one senior perinatal pathologist review all histopathological slides, we were striving to minimize this problem. In study II, the pathologists examined a portion of the placentas together, in order to establish common criteria and reach consensus on the histological diagnosis.

6.1.3.2   Missclassification of PE diagnosis

In the first studies, all diagnoses were verified in the clinical reports by collecting data on blood pressure levels, laboratory findings and symptoms. In the third study, we collected the cases from the hospital's diagnostic database. A validation of the coding in the diagnostic database was done by reading medical records of >10% of the patients from each year. All of the patients were correctly diagnosed with PE. Among them, 11.3% had mild PE, 88.7% severe PE and additionally, 24.2% HELLP syndrome.

6.1.3.3   Confounding

A confounder is an additional variable related to both the exposure and the outcome, therefore influencing the study on association between exposure and outcome. One way to minimize the effect of a confounder is to match participants in the different groups in regard to the confounder. However, it can be difficult to find pairs and the sample size can be reduced. Another possibility is to make a statistical analysis in which you include the confounder and therefore adjust for its effect.[200]

In this thesis, the most obvious confounder is the gestational week. We chose to include the gestational week in the statistical analyses. Another confounder that we identified in the last study was presence of PE. Compared to the other studies, this sample consisted of an extremely premature population in which only some were complicated by PE.

With a smaller study group, fewer variables can be included in the statistical analysis. This accounts for both outcome variables and confounders. The recommended number of variables is 1 per 10 cases.[198] Depending on the sample sizes in our studies, we have either included only the gestational week in addition to one outcome variable, the gestational week in addition to several outcome variables or several possible confounders in addition to outcome variables.

6.1.3.4   Selection bias

Selection bias is present when exposure and outcome are related to the fact that a case is included in the study or not, or related to which study group the participant belongs to.[200]

In study I, 157 patients from Karolinska University Hospital Huddinge were included from the years 2000-2007. Approximately 1300 singleton PE cases were admitted at the hospital those years, so only about 12% of these women were enrolled in the study. Originally, only the years 2000-2004 were included, but as there were too few cases in the mild PE group we also included mild PE cases from 2005-2007. By doing this, the mild PE group grew from 29 to 41 cases. During the study period, there was no routine for referring mild PE cases, which most likely explain the difficulty to collect enough cases, although mild PE is more frequent than severe[4]. However, there is a high risk of a selection bias regarding the mild PE group. As there was no routine for referring them, the obstetrician possibly made the decision to refer the mild PE placenta as a consequence of a complicated delivery or an affected newborn. We cannot disregard the possibility that our finding with few differences between mild and severe PE might be biased by the selection of mild PE cases, maybe skewed towards a more severe group.

In the second study, 117 out of approximately 1 000 PE cases (12%) during the study period at Karolinska University Hospital Huddinge were included. However, we only included severe PE cases and the 1 000 included both mild and severe, so the drop-out was probably less. Anyhow, the high frequency of drop-out may have given rise to a study sample which is not fully representative of the original population.

In the third study, 544 placentas out of approximately 2800 (almost 20%) delivered at Karolinska University Hospital Huddinge and Solna, were examined. The small, although larger than in study I and II, proportion of placentas examined can have caused a selection bias. As the routines for referring mild PE differed during the years, a selection was again probably towards a severe PE population. First in late 2007, the routine for referring changed to include also mild PE placentas. Also during the years when both mild and severe PE routinely were sent for examination, a selection towards severe PE is likely as the proportion of mild PE cases was small also in those years. In addition, there might be a selection bias also within the mild PE group, as explained above in study II. However, this study correlated placental pathology, and not severity of the disease, with outcome. The proportion of examined placentas differed depending on year and hospital. In general, the referral rate grew from 9% to 27%. In the last study year, as many as 37% of the placentas from Karolinska University Hospital Huddinge were sent for examination.

However, the number of examined placentas was even greater in the last study. Almost 80% of all singleton infants born in gestational week 22-27 in Stockholm during the study period were included, which is a very high level and a strength of study IV.[207]

6.2 FINDINGS AND INTERPRETATIONS

An abnormal transformation of the spiral arteries, illustrated by *decidual arteriopathy* was associated with referral of the infant to neonatal care (**study III**). Although pathognomonic for PE, with the exception of fetal growth restriction without PE[19], no other relationship could be found. Our interpretation is that the sampling error, when not examining placental bed biopsies, explains the lack of findings related to

arteriopathy. We believe that the true frequency of decidual arteriopathy is much larger in our material than what we found.

Accelerated villous maturation was the most common finding in our PE materials. Although it cannot be adequately evaluated in the term placentas, it is likely that the villi of some term placentas also matured in advance. In **study III**, we found that placentas showing accelerated villous maturation were more likely to have a normal blood flow in the umbilical artery, showed a tendency to be inversely related to IVH ≥grade 3 and more often resulted in a live born infant. Conversely, others have shown immature villi to be related to fetal death.[64, 208] In **study IV**, the inverse relation between accelerated villous maturation and fetal death could not be confirmed, but this association has recently been found in another preterm population born before 32 weeks' of gestation.[209] It could be that deaths in even earlier gestations, such as in our cohort born before 27 weeks' of gestation, have causes not affected by villous maturation. In our cohort, fetal thrombosis was the strongest histopathological predictor of IUFD.

In the last study (**study IV**), accelerated villous maturation was related to a better Apgar score and showed a tendency to be related to better language development at 2.5 years of corrected age. In the absence of intervillous thrombosis it was also associated with a lower risk for neonatal mortality. In none of the studies, accelerated villous maturation was associated with adverse outcome of the infant or child. We speculate that accelerated maturation of the placental villi is a response to a hypoxic environment, and that it is a successful compensating mechanism leading to a better outcome of the infant. Maybe a child's maturing ability is related to the maturing ability of the placenta as a consequence of a genetic predisposition, and in that case, the placenta mirrors the child's future development.

Smoking is, in contrast to all other cardiovascular risk factors, associated with a decreased risk of PE.[210] Accelerated villous maturation can further be seen in placentas from mothers who smoke.[13] Our finding with better outcome in the presence of accelerated villous maturation makes us wonder if the presence of accelerated villous maturation could have any role in the protective effect of smoking.

Less surprising and mainly in accordance with previous studies[13, 19, 24, 140], the better recognized hypoxic sign, *infarction,* was related to several adverse perinatal outcomes such as abnormal umbilical artery blood flow, lower umbilical artery BE, SGA-infants and IUFD (**study III**). In the last study (**study IV**), we could also find an association with CP, which is also in agreement with previous studies on macroscopically diagnosed infarctions.[27, 28] Previous studies, that have shown associations between placental pathology and CP, have only diagnosed infarctions macroscopically. They most likely did not see the smallest infarctions and therefore mainly included the more extensive ones, influencing the infant to a greater extent.[27, 28] Nonetheless, the risk of misclassification of infarction is high without a microscopic investigation[33] and our study, which includes a microscopic verification of the macroscopic finding, confirms the relation between placental infarctions and a diagnosis of CP.

We could not find an association to development outcome, which could be explained both by the lower frequency of infarctions, as the last population was not a PE-population, and by the fact that we could not estimate the amount of infarction in that study. The cohort came from all hospitals in Stockholm with differing practice in examining the placentas and an estimation of the amount of infarction was not always performed. Small infarctions are common and not associated with complications in an otherwise normal placenta.[19] In contrast to the microscopic signs that were reexamined, the macroscopic features, such as amount of infarction, were gathered from the pathology reports. Furthermore, in a population born before gestational week 28, the cause of growth restriction is more likely of different origin than PE, thus not necessarily related to infarctions.[24] In this population, we found instead a relation between SGA and fetal thrombosis, discussed below.

A consequence of poor blood flow from the mother to the placenta and fetus could be growth restriction of both fetus and placenta. It is well known that the frequency of a low *placental weight* is more common in a PE-population.[13] However, recently also heavier placentas have been reported in PE at term.[35] Redman et al have suggested that the size of the placenta might matter as a larger placenta could release more debris, which could explain why multiple pregnancies are associated with an increased risk of PE.[73]

In **study III**, we found that both a low and a high placental weight in relation to gestational week were related to major neonatal morbidity. DM is associated with large placentas[13], but in our study, none of the women with a large placenta had DM. When we explored the major neonatal morbidities separately, we saw that a low placental weight was associated with NEC, whereas a high weight showed a trend to be related to morbidities such as cystic PVL and IVH ≥ grade 3.

Interestingly, although the occurrence of accelerated villous maturation was high (73.1%) in our cohort, none of the preterm large placentas (n=12) showed any signs of accelerated maturation. We speculate that the placenta may compensate for the inadequate blood flow to the fetus in different ways, either by maturing the villi, or by growing faster. As mentioned earlier, acceleration of the villi seems to be a successful compensating mechanism. In contrast, a large placenta was related to major neonatal morbidity and specifically to severe morbidities of the brain. On the other hand, accelerated villous maturation showed a tendency to be associated with a lower incidence of IVH ≥ grade 3. So, from the maturing brain's point of view, an acceleration of the villi would be preferred to a large placenta.

The relation between abnormal placental weight and major neonatal morbidity was not confirmed in **study IV**. Perhaps the placental weight in the PE-population was more important than in the extremely premature population, in which chorioamnionitis was a more common finding.

Fetal thrombosis, a vascular lesion on the fetal side of the placenta, is not associated with PE.[40] While only 7.0% of the PE-placentas in **study III** had fetal thrombosis, it was found in 30.6% of the extremely premature placentas in **study IV**. In study IV, fetal thrombosis was related to SGA-infants, IUFD and also showed a tendency to be

associated with adverse cognitive function at 2.5 years of age. These results are in concordance with previous studies.[40, 42, 43] In study III, fetal thrombosis was related to admission for neonatal care in the model analysis, but the CI of the OR included 1.00, probably as the frequency of fetal thrombosis in that material was too small. In the last study, where all were born before gestational week 27, all were obviously admitted to neonatal care unit.

Our findings regarding *chorioamnionitis* differs between **study III** and **IV**, which can partly be explained by the different populations and the different patterns of pathology. Several studies have previously investigated the relations between chorioamnionitis and fetal outcome and development.[50]

Whereas accelerated villous maturation is a positive sign from the infant's point of view, it might not benefit the mother. Both mild and severe PE showed significantly more accelerated villous maturation than the control group (**study I**). As severe PE is diagnosed in the presence of SGA, in an otherwise mild PE, we decided to present subgroups of the severe PE group in this thesis. As is seen when stratifying after SGA and AGA, descriptive data on accelerated villous maturation shows a higher frequency of accelerated maturation when the mother had high blood pressure or proteinuria or additional symptoms fulfilling the criteria for severe PE.

We speculate, that the more hypoxia, the more common is accelerated villous maturation. The lack of normal placentation leads to an inadequate blood flow and unsatisfactory oxygen supply from the maternal side of the placenta to the fetal side. The hypoxic environment has shown to stimulate the placental villi, on the fetal side of the placenta, to secret sFlt-1[211], a soluble vascular endothelial growth factor receptor, which spreads to the maternal circulation and could explain some of the maternal symptoms[74]. A previous study[212] has shown that the level of sFlt-1 was significantly increased in the maternal blood of both mild and severe PE compared to controls, but there was only a trend towards a difference between mild and severe PE. However, a study by Taché et al[213] showed that overexpression of sFlt-1 in the placenta strongly correlates both with the severity of hypertensive disease and accelerated villous maturation.

Nonetheless, the frequency of ≥5% infarction significantly differed between mild and severe PE, as compared to the other pathological features (**study I**). Furthermore, all pathology that was more common in severe PE compared to the control group was also significantly more common in mild PE. This is not supporting the common notion that mild PE is not the same syndrome as severe PE, but only part of a normal physiological development in pregnancy. Also supporting the seriousness of a mild disease, Koopmans et al found that induction of labor beyond 37 weeks' gestation improved maternal outcome also in mild PE.[141]

Our results could indicate a similar underlying pathology in mild and severe PE, and that different individuals answer in diverse ways to this pathology. Let us assume that the progress of pathology is as follows: decidual arteriopathy – accelerated villous maturation – infarctions ≥5%, probably with acceleration and infarction developing in parallel. Perhaps the pathophysiological processes are the same until infarctions

develop, explaining why this variable showed differing frequencies. Could cardiovascular risk factors play a role here? The metabolic syndrome has previously been associated with PE[4, 214] and it is possible that persons prone to develop cardiovascular disease might also be more likely to develop placental infarctions of ≥5% and severe PE.

No other studies have primarily examined extent and type of placental pathology in relation to severity of disease, but a study by Moldenhauer et al[68] on placental pathology in relation to gestational age has shown more pathology in earlier gestational weeks. As our severe PE group is of a younger gestation than the mild group, we think that this supports our finding that infarctions are more common in the severe group. Still, Moldenhauer et al presented high frequencies of pathology in all gestational weeks, in comparison to the control group, also supporting our hypothesis that mild PE is part of the PE spectrum and not a normal physiological development. We do not doubt that there is an impact of gestation, but our interpretation is that both gestational week and the severity of disease is related to the extent and type of pathology.

On the other hand, HELLP, which is a commonly accepted phenotype of PE, although with many differences in symptomatology, was shown to have a discordant histopathology (**study II**). In the PE group, we found more typical PE signs of abnormal uteroplacental blood flow, such as intervillous thrombosis, infarctions ≥5% and abruption. In patients with PE, the vascular resistance has been found to be reduced, whereas the vascular reactivity in HELLP patients did not differ compared to controls.[215] This indicates that PE without HELLP might be a more vascular disease, which is also supported by our findings with more infarctions, thrombosis and abruption in the PE group.

If PE is a vascular disease, leading to a chronic abnormal uteroplacental blood flow affecting growth of both fetus and placenta, the HELLP syndrome might be of a more toxic and acute nature. This could explain why pathology had not yet developed in the HELLP placentas and why fetal and placental growth was not affected to the same degree. This is supported by our finding of the shorter time between diagnosis and delivery in the HELLP patients.

Previously, a study by Smulian et al[216] on placental pathology in PE and HELLP patients showed a difference in placental abruption, in parallel with our results. However, they did not find any other pathological differences between the groups, which could be explained by their smaller study group. Another study[217] did not find any difference in abruption either, but used less strict diagnostic criteria.

However, in the comparison between PE and HELLP, it must be remembered that many of the HELLP patients also had hypertension and proteinuria of severe level and obviously have pathology related to that. Whether HELLP and PE are different diseases often affecting the same persons or rather part of the same disease is still unclear.

7 CONCLUSIONS

It is debated whether mild PE is a physiological development of a normal pregnancy or part of the PE spectrum. Our results, with similar placental pathology in mild and severe PE in relation to controls, as well as the correlation between amount of pathology and the severity of PE **(study I)** point towards one disease originating from the placenta and giving rise to mild as well as severe PE, probably depending on the individual and the timing.

The etiology of PE is still unknown, but the placenta is regarded as a central organ in the pathogenesis. As the placental pathology in severe PE with and without HELLP differed **(study II)**, we conclude that the pathophysiological mechanisms in PE and HELLP might differ.

Accelerated villous maturation was associated with normal blood flow in the umbilical artery in the PE population **(study III)**, indicating a possible compensating mechanism for a hypoxic environment. On the other hand, an abnormal umbilical artery blood flow was related to low placental weight and infarctions ≥5%.

Typical placental pathology in PE was further associated with perinatal and neonatal outcome of the infant **(study III)**. Infarctions ≥5%, abnormal placental weight and decidual arteriopathy were related to different adverse outcomes, whereas accelerated villous maturation was inversely related to IUFD, once more suggesting a protective mechanism.

In an extremely premature population, relations between placental pathology and perinatal and neonatal outcome could also be found **(study IV)**, but the pattern was slightly different. Fetal thrombosis was related to IUFD and SGA. Furthermore, low placental weight was associated with IUFD. Also in this study, accelerated villous maturation was associated with a positive outcome: better Apgar score.

As relations could be found between placental pathology and neonatal outcome both in the PE-population **(study III)** and the extremely premature population **(study IV)**, we conclude that a careful pathological examination of the placenta without delay may add important prognostic information on the neonates at risk.

We found a relation between placental infarction and CP, but no conclusions could be drawn regarding associations between placental pathology and development at 2.5 years of age **(study IV)**. However, we did see that accelerated villous maturation tended to be associated with better language development, fetal thrombosis tended to be related to adverse cognitive development and low placental weight tended to be related to worse motor development. Larger studies must be conducted in order to confirm those tendencies.

8 FUTURE PERSPECTIVES

Severe PE obviously covers several different disease entities: women with HELLP syndrome, PE-patients with moderate hypertension and proteinuria but with the presence of fetal growth restriction and lastly, women with severe hypertension and proteinuria both with and without impact on the fetus. Further, non-PE pregnancies complicated by late spontaneous abortions and fetal growth restriction have been related to abnormal development of the spiral arteries, which is associated with PE. It will be intriguing to elucidate why some patients develop severe maternal symptoms and others no maternal symptoms, but growth restriction or even spontaneous abortion or IUFD.

It would also be interesting to study the HELLP syndrome further. Many patients, but not all, do not fulfill the criteria for PE without their HELLP diagnosis. A characterization of HELLP patients without hypertension and proteinuria could add knowledge in the understanding of PE in relation to HELLP.

Studying the developmental outcome should be done in a larger material and also at a longer term. If such studies confirm what we showed in this thesis, also studies on intervention, for example hypothermia, in patients with severe placental pathology, but otherwise not eligible for treatment, could be considered.

All relations that we could find regarding accelerated villous maturation were positive findings. It would be interesting to investigate what makes some placentas "accelerate" their villi and others not. Also, can external factors, such as high altitude or smoking, stimulate the villi to accelerate and influence the infant positively?

Maternal factors influencing the risk of PE could be studied further. Smoking is shown to protect against PE. The mechanisms behind that should be studied and could add knowledge both regarding pathophysiological mechanisms in PE and in the search for a treatment. Also, overweight and obesity are related to PE. Women are becoming more obese and it is essential to study this growing group further.

9 SAMMANFATTNING PÅ SVENSKA

9.1 BAKGRUND

Preeklampsi (PE) är vad man i folkmun kallar havandeskapsförgiftning, vilket drabbar 2-7% av gravida kvinnor i världen. Både modern och barnet påverkas av sjukdomen och PE är en av de vanligaste orsakerna till att gravida kvinnor och deras barn blir sjuka och dör. I västvärlden är PE anledningen till 15-20% av mödradödligheten.

När kvinnan blir gravid och moderkakan (placentan) bildas ska blodkärl som finns i livmodern omvandlas så att blodflödet till barnet blir optimalt. Omvandlingen av blodkärlen uteblir dock vid PE och istället får kärlen ett avvikande utseende, en sämre funktion och moderkakan drabbas av ett försämrat blodflöde.

I moderkakan ser man oftare fynd som kallas för decidua arteriopati, infarkter, ablatio och intervillösa tromboser. Decidua arteriopati är det man ser när kärlen inte utvecklats på rätt sätt, såsom ovan beskrivits. Ytterligare väger ofta moderkakan mindre.

Kvinnorna drabbas framför allt av olika grader av högt blodtryck samt läckage av äggvita i urinen. Om man inte åtgärdar sjukdomen kan den leda till ett kramptillstånd som kallas eklampsi. Exakt hur och varför PE uppstår är dock ännu inte helt klarlagt. Det enda sättet att bota sjukdomen är att förlösa barnet och moderkakan, vilket förklarar tillfrisknandet efter förlossningen. Sjukdomen kan dock uppstå redan i graviditetsvecka 20, då barnet ännu inte skulle klara sig utanför mammans mage. Sveriges omfattande mödrahälsovård syftar till stor del till att hitta kvinnorna som utvecklar PE.

Sjukdomen kan ytterligare kompliceras av att mödrarna får något som kallas för HELLP syndrom, vilket kännetecknas av illamående, buksmärta och uppkastningar. Vid HELLP blir blodplättarna färre, blodkropparna går sönder och leverenzymerna stiger. Idag klassificeras HELLP som en sorts PE, trots att symtomen skiljer sig. Många patienter har såväl HELLP- som PE-symtom, men en del patienter har bara de ena symptomen.

PE brukar delas in i en mild och en svår variant, beroende på graden av symptom. Vid högre blodtryck, än mer läckage av äggvita i urinen, tillägg av HELLP syndrom och tillväxtrubbning hos barnet klassas sjukdomen som svår. Det är debatterat huruvida en patient med mild PE har PE eller om mild PE bara är en naturlig utveckling av graviditeten. Ovan nämnda klassifikationsproblem kan vara en bidragande orsak till varför man inte lyckas komma fram till vad sjukdomen beror på.

Barnen till kvinnor med PE växer ofta sämre och föds ofta för tidigt, vilket i sig är en riskfaktor för sjukdom och död. En låg födelsevikt har dessutom visat sig vara kopplad till hjärt- och kärlsjukdomar i vuxen ålder. Tidigare studier har beskrivit barnets hälsa hos PE-graviditeter, men få har letat efter samband mellan fynd i moderkakan och barnets sjukdomar och deras dödlighet. Det finns dock fynd i moderkakan som har visat sig ha samband med diverse sjukdomar hos barn.

9.2 FRÅGESTÄLLNINGAR

1. Finns det någon korrelation mellan patologin i moderkakan och mammans svårighetsgrad av sjukdom?

2. Är det någon skillnad i moderkakspatologi och frekvens tillväxt-hämmade barn hos svåra PE-fall med respektive utan HELLP syndrom?

3. Förekommer något samband mellan moderkakans patologi vid PE och barnets sjuklighet och dödlighet under nyföddhetsperioden?

4. Förekommer något samband mellan moderkakans patologi och barnets sjuklighet och dödlighet under nyföddhetsperioden hos mycket för tidigt födda barn?

5. Har moderkakans patologi någon relation till barnets utveckling vid 2.5 års ålder hos mycket för tidigt födda barn?

9.3 STUDIE I

Materialet i denna studie består av 157 kvinnor med PE från Karolinska Universitetssjukhuset Huddinge. Av dessa hade 40 mild PE och 117 svår PE. Ytterligare rekryterades en kontrollgrupp med 157 kvinnor från graviditeter utan problem med högt blodtryck. Kontrollerna var matchade avseende graviditetsvecka.

Kliniska data samlades från journaler. De nyfödda barnen diagnostiserade som SGA (små i relation till graviditetsvecka) när födelsevikten avvek mer än 2 standard deviationer enligt en skandinavisk respektive holländsk tillväxtkurva. En senior patolog eftergranskade samtliga mikroskopiska glas och var då blindad för klinisk data. Makroskopiska data hämtades från patologi-rapporten.

Moderkakans vikt samt övriga patologiska variabler jämfördes mellan grupperna. Statistiska analyser gjordes med multipel regression, Chi-square-test samt T-test. I regressionsanalyserna justerades resultaten för graviditetsveckan när den visade sig ha en inverkan på utfallet.

De patologiska fynden decidua arteriopati, accelererad villusmognad och andel infarkter i moderkakan ökade med svårighetsgrad av sjukdom hos mamman. Utöver att det var mer patologi vid svår PE, var patologin vid mild och svår PE liknande i jämförelse med kontrollgruppen.

9.4 STUDIE II

Denna retrospektiva studie inkluderar 178 kvinnor med svår PE från Karolinska Universitetssjukhuset Huddinge eller Free University Medical Center Amsterdam. Av dessa hade 96 svår PE utan HELLP och 82 fall hade svår PE med HELLP.

Kliniska data om patienterna och patologi-data hämtades från journaler och patologi-rapporter. Födelsevikten klassificerades såsom i studie I.

Barnets födelsevikt, moderkakans vikt samt övriga patologiska fynd jämfördes mellan de två grupperna. Statistiska analyser gjordes med logistisk regression, ANCOVA, Chi-square test, Fisher's exact test, Mann-Whitney-U-test och T-test beroende på vilken variabel som studerades. När graviditetsveckan visade sig ha en inverkan på utfallet inkluderades den i analysen som en kovariat.

De patologiska fynden infarkter, intervillös trombos och ablatio var vanligare i PE-gruppen utan HELLP. En högre moderkaks-vikt var vanligare i PE-gruppen med HELLP. PE-gruppen utan HELLP hade en högre frekvens av små barn i relation till graviditetsvecka.

9.5   STUDIE III

Denna studie inkluderar 544 kvinnor och barn där modern haft PE, eklampsi och/eller HELLP på Karolinska Universitetssjukhuset Huddinge eller Solna.

Kliniska data hämtades från MFR (Medicinska födelseregistret), PNQ (Perinatala kvalitetsregistret) och journaler. Mikroskopiglasen eftergranskades av en patolog, som var blindad vad gällde utfall. Makroskopiska data hämtades från patologi-rapporterna.

Utfallsvariabler som inkluderas var neonatal mortalitet, Apgar-poäng, behov av sjukhusvård, barn födda SGA (små för gestationsåldern), allvarliga nyföddhetssjukdomar, blodförgiftning och behov av respiratorvård. Allvarliga nyföddhetssjukdomar inkluderade cystisk PVL (periventrikulär leukomalaci), IVH (intraventrikulär blödning) $\geq$ grad 3, NEC (nekrotiserande enterokolit), ROP (nyföddhets-retinopati) $\geq$grad 3 och/eller svår BPD (bronkopulmonär dysplasi).

Neonataldata korrelerades till typ av fynd i moderkakan. Multipla regressionanalyser användes, men även enklare statistiska metoder såsom Fisher's exact test. I de multipla regressionanalyserna inkluderades graviditetsveckan när det var lämpligt.

En avvikande moderkaks-vikt var relaterad till allvarlig nyföddhetssjukdom hos barnet. En låg vikt ökade risken såväl som en hög. Fyndet accelererad villusmognad var relaterad till minskad frekvens dödfödda barn. Fyndet decidua arteriopati var associerat med ökat behov av vård på sjukhus för barnet. Infarkter i $\geq$5% av moderkakan var relaterat till dödfödda barn och SGA-barn. Några typiska fynd i moderkakan i relation till dödlighet under nyföddhetsperioden hittades inte.

9.6   STUDIE IV

Materialet i denna studie består av 167 barn och mödrar med förlossning före graviditetsvecka 27 i Stockholm län. Av dessa var 62 dödfödda och 38 dog inom en månad. Vid 2.5 års ålder undersöktes 59 med avseende på utveckling.

Kliniska data hämtades från PNQ och från journaler. Alla mikroskopiglas av moderkakorna eftergranskades av en patolog som var blindad för utfallet. Makroskopiska data hämtades från patologi-rapporten.

Utfallsvariabler som studerades var SGA-barn, allvarliga nyföddhetssjukdomar, blodförgiftning samt behov av behandling av kvarstående duktus arteriosus. Allvarliga nyföddhetssjukdomar definierades såsom i studie III. Data om allvarlig neuromotorisk (CP) eller neurosensoriskt (allvarlig synnedsättning och dövhet) handikapp analyserades. Allvarlig synnedsättning definierades som blindhet eller förmåga att endast se ljuset från en lampa. Allvarlig hörselnedsättning definierades som en oförmåga att höra trots hörapparat. Kognitiv, språklig och motorisk utveckling vid 2.5 års ålder bedömdes med hjälp av Bayley-III utvecklings-tester och jämfördes med normala resultat hos skandinaviska barn.

Neonataldata samt neurologiska data och utfall vid 2.5 års ålder korrelerades till fynd i moderkakan. De olika statistiska metoderna som användes var logistisk regression, som inkluderade olika confounders som visade en inverkan på utfallet, stegvisa statistiska metoder, liksom ANCOVA-analyser (analys med kovariater) samt enklare analyser som Fishers exact test, Mann-Whitney U-test och T-test.

Accelererad villusmognad var associerad med bättre Apgar-poäng. Fetala tromboser och en låg moderkaks-vikt var relaterade till både dödfödda barn och SGA-barn. Infarkter i placentan var associerade med en CP diagnos hos barnet. Accelererad mognad visade en tendens att vara relaterat till bättre språklig utveckling, fetala tromboser till lägre kognitiv funktion och en låg vikt till sämre motorisk utveckling.

9.7 SLUTSATSER

- Att patologin vid mild och svår PE liknade varandra samt skiljde sig från kontrollerna indikerar att de underliggande mekanismerna kan vara desamma vid mild och svår PE. Detta talar emot att mild PE är en naturlig utveckling av graviditeten.

- Att patologin i moderkakorna från PE med respektive utan HELLP skiljde sig skulle kunna tyda på att PE och HELLP är olika sjukdomar med olika etiologi, men som ofta drabbar samma patienter.

- Hos såväl barn födda av PE-kvinnor som extremt för tidigt födda barn finns det fynd i moderkakan som korrelerar med det neonatala utfallet. Hos dessa barn skulle undersökning av moderkakan kunna förbättra riskbedömningen av det nyfödda barnet.

- Infarkter i placentan verkar öka risken för att barnet drabbas av CP. För att några slutsatser ska kunna dras avseende associationer mellan fynd i moderkakan och barnets utveckling vid 2.5 års ålder behövs större studier. De tendenser som framkom tyder på att flera samband mycket väl skulle kunna finnas.

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