General Discussion

8.1. Prediction of PE: experimental biomarkers and the need for a clinical useful screening test

As described in this thesis, PE is a complex and severe pregnancy disorder, which can have lifelong consequences for both mother and child. In the last decades, prediction of this disorder has extensively been studied in order to make risk stratification and prevention possible. Identification of women at risk of PE is the first step to effective intervention and prevention (20, 23).

The main problem, which makes it difficult to perform these studies, is the fact that we do not know the exact etiology of PE yet and in addition to this the heterogeneous character of the syndrome with different phenotypes: early- versus late- onset PE, severe versus mild PE as well as superimposed PE and HELLP syndrome. It makes it even more complex that different phenotypes of PE may simultaneously occur in one woman and sometimes overlap each other. The different phenotypes are likely to reflect different underlying biological mechanisms (4). For example, in this thesis we showed clearly the difference between early- and late onset PE. Investigating biomarkers with poor definitions of phenotypes, results in low predictive values. In PE it is not one single entity that can predict the disorder. Multiple factors are responsible for the onset of clinical disease (24, 234). In this thesis we showed that in the development of a predictive test, it is important to focus on combination models, in which multiple laboratory-based biomarkers, and possibly also ultrasound markers, have to be combined. Ideally, an easy-accessible, cheap, laboratory-based screening test with a high predictive value will be developed.

Another problem is that, because of the relatively rarity of the disorder, many studies are underpowered. For this reason there is urge for well-designed, prospective, big cohort studies. Collaborative initiatives in international research consortia may make these extensive studies possible. One example is the IMPROvED (Improved Pregnancy Outcomes by Early Detection) study, which aims to develop a clinically robust predictive blood test for PE, by recruiting 5000 healthy, nulliparous women in five European countries (77).

A third important issue in the development of a screening tool for PE is that this test has to be clinically relevant and cost effective. Incorrect risk stratification may have ethical consequences and lead to inappropriate medication or pregnancy surveillance. The WHO criteria by Wilson and Jungner describe different requirements for screening (79) (figure 1). Several of these points are not yet true for the condition of PE but, criterion 7 might be the most essential point. Although much research has been performed to elucidate the etiologic pathway, the natural history of PE, including development from presymptomatic to clinical disease, is not yet fully understood. Also, criterion 4 is difficult to achieve for PE. Of many proposed biomarkers, including the protein and epigenetic markers investigated in this thesis (i.e. calcyclin, HSP90, clock genes and protein AMBP), it is not clear whether these factors are a cause or a consequence of the disorder. Assuming that the onset of PE starts around the period of early placentation, we have to search for predictive biomarkers very

early in pregnancy (1). However, at that time the question is whether foetal and placental substances are already present in the maternal circulation. Spiral artery remodeling has not taken place yet and placental plugs are still present as a barrier between the circulation of mother and child. One might not find any differences in the blood of a woman with a healthy pregnancy outcome compared to a woman that will suffer from PE later on in pregnancy. Many biomarkers which are suggested as predictive markers, e.g. oxidative stress and inflammatory factors, may be released in the maternal circulation after the onset of the pathologic process: 'when the damage is already done'. Therefore, it is vital to study PE very early or even before pregnancy.

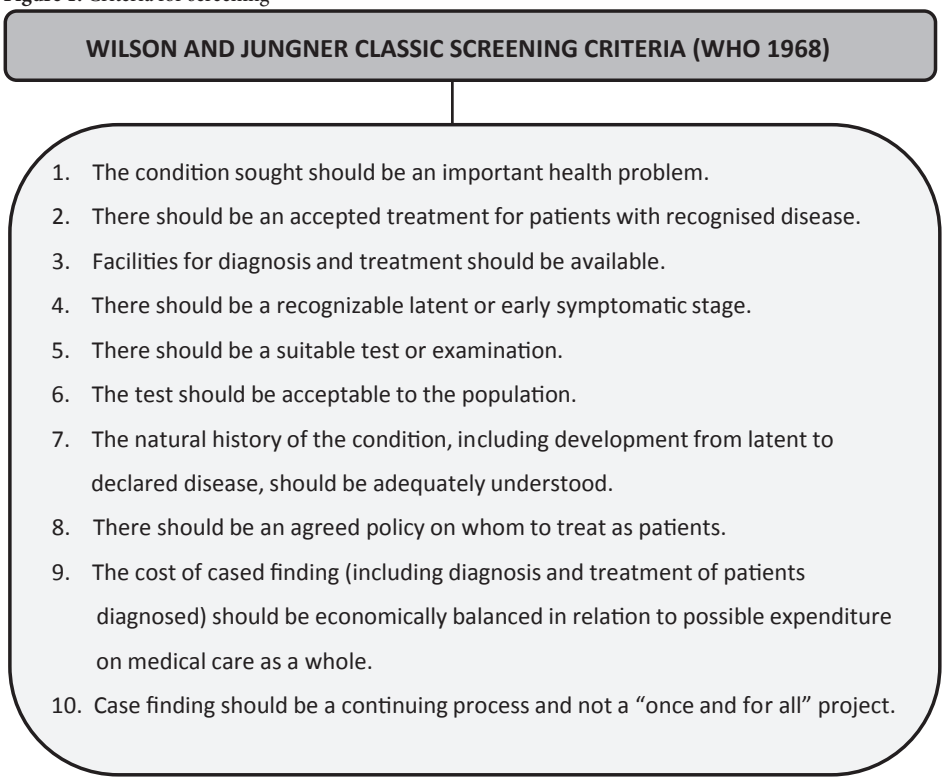
Foetal and placental epigenetic programming may play a main role in this pathophysiologic process. In this thesis we present the results of two epigenetic studies, in which we investigated candidate genes in placental tissue, umbilical cord white blood cells and endothelial cells of the umbilical cord after birth. Hence, still we cannot say when and why these alterations initially occurred; as a cause or consequence of the disorder. However, alterations in epigenetic programming may be of great importance for the health of the offspring. In line with the DOHaD paradigm (14) it will be interesting to study whether these DNA methylation markers were already present in the parents' epigenome and also to investigate how they affect the children's health risk in future life.

8.2. Adaptation of the mother's brain during pregnancy and PE

Although we are currently not able to predict or prevent PE, it is as important to monitor the mother's health during clinical disease in order to prevent severe complications such as eclampsia (3). Since the introduction of administration of magnesium sulphate to women at risk for convulsions, the occurrence of eclampsia has decreased dramatically (235). However, already in an earlier stage, before the occurrence of convulsions, the mother's brain may be affected by (severe) pre-eclampsia. Previous studies showed that women with PE showed alterations by using neuroimaging techniques and neurophysiology tests (8, 17, 189, 236, 237). In addition to this, women with a history of PE have a higher risk of cerebrovascular damage later on in life and report abnormal cognitive symptoms after pregnancy (186-188).

In this thesis we reported two brain studies in women with (severe) PE. In the first study we found a clear difference between the protein profiles of cerebrospinal fluid from patients with PE and normotensive pregnant women at delivery. As described in the introduction, many factors, such as hormones, cytokines, growth factors, pass the foetal-placental unit and are secreted into the maternal circulation. In PE these factors differ from normotensive pregnancy and may become toxic for the maternal endothelium. Highly vascularized organs, in which the endothelium plays an important role, suffer the most from this problem. This may be an explanation of the fact that in PE mainly these organs are affected (liver, kidneys, brain and placenta). It also fits in the hypothesis that an altered BBB permeability plays a main role in cerebral complications (9, 11). Even during normotensive pregnancy

Figure 1. Criteria for screening



the BBB may already be more vulnerable for disruption, because of the changed vascular state of the mother. Previous research showed that pregnancy caused an significant increase in BBB permeability following acute hypertension in rats (198).

We found free haemoglobin in CSF of mothers with normotensive pregnancy and PE, which is a remarkable finding. This resulted in the hypothesis that there might already be an increased (physiological) BBB permeability during normal pregnancy. In women with PE CSF concentrations of protein AMBP were significantly higher. Protein AMBP is a precursor of A1M, a scavenger of toxic free haemoglobin. We hypothesize that when the brain is experiencing toxic effects of free haemoglobin during the pathologic process of PE, protein AMBP will be upregulated. This is in analogy to general processes in the human body; during an infection the immune system is activated and CRP-levels and leukocytes of an infected person will be higher than those of a healthy person. In conclusion, pregnancy itself makes the brain more vulnerable to cerebral complications, which is then aggravated in PE when the brain is experiencing toxic effects and protective proteins such as A1M are upregulated. Previous studies also demonstrated A1M in serum to be a marker for PE. Increased expression of foetal haemoglobin, which leaks over the placental barrier into the

maternal circulation, results in oxidative stress and endothelial damage (200). Free haemoglobin might cause damage to the brain in a similar way as described for the placenta-blood barrier. In this thesis we were not able to prove that this free haemoglobin was of foetal origin. Future research needs to elucidate how the BBB exactly functions during human pregnancy and how this might be detrimentally affected by PE.

In our second brain study, we investigated visual evoked potentials (VEP) during normotensive pregnancy and PE. Normotensive pregnant women had significantly shorter latencies during pregnancy compared to their postpartum measurements. Pregnancy is a state with higher neuronal excitability and a lower seizure threshold (194). From the results of this study we hypothesize that normotensive pregnant women experience a certain physiological adaptation of the brain during pregnancy, while this adaptation fails in women with PE.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

This thesis has increased our knowledge about pre-eclampsia and brain related complications. Relatively new and experimental techniques have been used to study this complex pregnancy disorder. The difficulty in finding a clinical useful predictive screening test for PE, using laboratory-based markers, was showed. Existing hypotheses have been tested and new hypotheses and questions arise from this thesis. Pregnancy is a unique state of the human body in which major adaptations, mainly in the vascular system, occur. These adaptations seem to fail in PE, causing damage to several organs. This fits in the existing idea that pregnancy is a 'stress test' for the mother's vascular system. Specific for the maternal brain we found evidence for an altered BBB and an altered neurophysiological function of the visual system. Future research should focus either on well-designed, clinical trials in which we test the usefulness of screening tests, and on the other hand on high quality basic and translational studies, in which the exact mechanisms occurring during placentation and during the clinical onset of disease will be further elucidated. We also argue that women with severe PE should be admitted and monitored at obstetric critical care units in tertiary perinatology centers. These settings provide the opportunity to optimize clinical care for these women and to collect comprehensive data of cardiovascular and cerebral functions during severe PE.