Endothelial pathology in preeclampsia

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Chapter 7

Summary and general discussion

SUMMARY

As outlined in the introduction of this thesis, preeclampsia is a potentially devastating pregnancy complication with a complex, multifactorial aetiology. The risk factors lie within the genetic, vascular and immune domains. Genetic risk factors reported previously fall into two distinct categories: coagulation and vascular maintenance on one hand, and immunology on the other. Acquired contributing factors comprise vascular and immunologic components as well, with pre-existing diabetes, hypertension, obesity and older maternal age falling in the first, and oocyte donation, autoimmune diseases, nulliparity and new paternity falling into the latter category, respectively. These challenging vascular and immunologic states supposedly contribute to impaired placental development, placental oxidative stress and production of anti-angiogenic factors and subsequent systemic endothelial dysfunction. The complex interplay between these three pathways leading up to endothelial dysfunction in preeclampsia (coagulation, inflammation and angiogenesis) is studied in this thesis.

Chapter 2 - genetic variants in preeclampsia

Preeclampsia has a clear familial component, suggesting that the condition may be partly attributable to genetic susceptibility. Within the genetic susceptibility, clues on the causal pathways of the syndrome might remain. However, many genetic association studies on preeclampsia have been performed, but they have yielded inconsistent results. Therefore, **chapter 2** focuses on identifying the pooled effect of each genetic variant that is reproducibly associated with preeclampsia through meta-analyses. We selected all genetic variants that were significantly associated with preeclampsia in an initial study and were subsequently independently reproduced in at least one additional study.

Seven variants remained significantly associated with preeclampsia following meta-analysis. These variants were in or near the following genes: angiotensin converting enzyme (ACE), cytotoxic T-lymphocyte associated protein 4 (CTLA4), coagulation factor 2 (F2), coagulation factor 5 (FV), lipoprotein lipase (LPL), and serine protease 1 (SERPINE1). Interestingly, four of these mutations (in F2, F5, and SERPINE1) are found within genes involved in the coagulation and fibrinolysis systems, reinforcing the association between inherited thrombophilia and preeclampsia. Two genes (ACE and LPL) are directly

involved in endothelial biology; ACE is a key regulator of blood pressure through activation of the renin-angiotensin-aldosterone system, and LPL facilitates lipid uptake in endothelial cells. Dysregulation of these pathways causes endothelial activation and a subsequent inflammatory response. Interestingly, these variants are also risk factors for developing cardiovascular disease, revealing that preeclampsia and cardiovascular disease have shared genetic risk factors. Many association studies have reported links between mutations in genes involved in the immune system and preeclampsia, but only a mutation in CTLA4 remained significant after meta-analysis.
These results reveal coagulation and inflammation as key elements in the genetic background of preeclampsia. Studying these pathways in depth might provide new insights on how placental dysfunction and the angiogenic imbalance of preeclampsia develop. However, the role of the genetic component in preeclampsia should not be overestimated: not all women with these mutations develop preeclampsia, and not all women who suffer from preeclampsia carry these mutations. Pre-existing endothelial damage or immunologically challenging pregnancies could provide the other 'hits' necessary for the development of placental dysfunction and the syndrome.[1, 2]

Chapters 3 and 4 - thrombomodulin and the angiogenic imbalance of preeclampsia
A possible link between endothelial damage and dysregulation of coagulation and inflammation as seen in preeclampsia could be thrombomodulin. This is an endothelium- and syncytiotrophoblast-bound protein that regulates coagulation, inflammation, apoptosis, and tissue remodelling. Thrombomodulin can be cleaved from the endothelium under inflammatory conditions, and in preeclampsia, levels of this non-functional soluble thrombomodulin are increased, indicating loss of functional thrombomodulin from the endothelium. Therefore, we hypothesized that thrombomodulin loss and subsequent loss of its mediating effects on coagulation and inflammation could be involved in preeclampsia. **Chapter 3 and 4** of this thesis are focused on the role of thrombomodulin in preeclampsia. First, we investigated placental thrombomodulin dysregulation and consequent downstream effects in the pathogenesis of preeclampsia; these experiments are described in **chapter 3**.
Thrombomodulin protein and mRNA expression were investigated in placentas from women with preeclampsia, from women with a normal, term

pregnancy and from women with a pregnancy complicated by intrauterine growth restriction matched for placenta- and birth weight. Both protein and mRNA expression were significantly decreased in preeclampsia as compared with both control groups, indicating loss of thrombomodulin production and abundance in the preeclamptic placenta. Further, thrombomodulin mRNA expression correlated with maternal body mass index and diastolic blood pressure in preeclampsia, suggestive of a link with the extent of vascular dysfunction.
The pathways mediated by thrombomodulin, including coagulation, inflammation, apoptosis and tissue remodelling, were investigated in this subsets of placentas. An increase in placental apoptotic cells was associated with preeclampsia. Thrombomodulin expression correlated positively with matrix metalloproteinase expression in preeclampsia, but not with fibrin deposits, inflammatory markers or the influx of inflammatory cells.
Studies in endothelial cells have shown before that VEGF is a stimulator of thrombomodulin expression.[3] Therefore, we hypothesized that the angiogenic imbalance of preeclampsia could be the cause of the low placental thrombomodulin levels found in our group of patients. Indeed, placental soluble Flt-1 mRNA expression correlated with decreased thrombomodulin expression. To further study the possibility of a causal mechanism, thrombomodulin mRNA expression was determined in VEGF-transfected trophoblast cell lines; VEGF induced significant upregulation of thrombomodulin mRNA expression in these cells.

Altogether, the experiments described in **chapter 3** stress the link between the angiogenic imbalance and endothelial pathology preeclampsia. Concurrently, they stage thrombomodulin as a new role-player in the development of placental and endothelial pathology in preeclampsia. In **chapter 4**, these mechanisms are investigated more in-depth in the organ most affected during preeclampsia, the kidney.
Glomerular thrombomodulin was studied in human patients with preeclampsia and deceased pregnant women without hypertensive complications as control subjects. Kidney thrombomodulin abundance was increased significantly in preeclampsia patients compared to pregnant control subjects. Interestingly, glomerular endothelial thrombomodulin expression was associated with parietal epithelial cell activation and nephrin

expression in podocytes, indicative of a protective effect on the glomerular filtration barrier.
Subsequently, we hypothesized that sFlt-1 might be responsible for this increase in glomerular thrombomodulin expression, but in vitro, sFlt-1 addition to human umbilical vein endothelial cells resulted in downregulation of thrombomodulin expression. Contrastingly, glomerular thrombomodulin abundance increased in mice treated with the anti-angiogenic factors sFlt-1 and sEng. Results from **chapter 3** hinted towards an association between obesity and thrombomodulin loss; therefore, glomerular thrombomodulin expression was investigated in porcine models for diabetes and metabolic syndrome; glomerular thrombomodulin was increased in these models as well.
In sum, **chapter 4** provides evidence for the hypothesis that angiogenic factors regulate renal endothelial thrombomodulin expression, in vitro and in vivo. Possibly, the increased endothelial thrombomodulin expression in preeclampsia indicates an attempt of glomerular endothelial cells to maintain cytoprotection during the challenging, anti-angiogenic environment of preeclampsia. Such a mechanism has been proposed before in glomerulonephritis.[4] Investigating pathways through which thrombomodulin expression is increased in endothelial cells could reveal clues to restore or prevent endothelial damage in the kidney. Interestingly, thrombomodulin expression is associated with increased expression of podocyte markers; this stresses the importance of cross-talk across the glomerular basement membrane, between endothelial cells and podocytes.

Chapter 5 - the interplay between endothelium and podocytes in preeclampsia
The role of this cross-talk in preeclampsia is investigated further in **chapter 5.** In this chapter, current literature concerning the kidney in preeclampsia was reviewed for studies concerning endothelium and podocyte biology and their cross-talk.
Early studies mainly describe endothelial cell swelling or 'endotheliosis', and the appearance of apparently bloodless glomeruli as trademarks of preeclampsia.[5] However, since endothelial pathology is not observed in all patients with preeclampsia and proteinuria, these lesions cannot explain the renal pathology in preeclampsia on their own. Podocyte foot process effacement in preeclampsia has been described as early as 1980,[6] but the

hypothesis for direct podocyte involvement in the pathogenesis of the syndrome had not been proposed until 2007.[7] Since then, numerous studies on the development of podocyte damage and the use of podocytes in urine as a diagnostic tool have been published.

The angiogenic imbalance in preeclampsia plays a key role in the development of both podocyte and endothelial damage in the glomerular filtration barrier and is presumed to be responsible for a glomerular *damage loop*. VEGF is a crucial survival factor for both cell types in the glomerular filtration barrier. Glomerular VEGF is predominantly produced in the podocytes; high levels of sFlt-1 prevent glomerular VEGF from binding to its receptors on the podocytes and glomerular endothelial cells. Consequently, podocytes get damaged and detach from the glomerular basement membrane, leading to even less available VEGF through diminished production. Endothelial cells, deprived of VEGF, get swollen and produce endothelin, which damages the podocytes even further.

An appropriate treatment for preeclamptic nephropathy might be established by interrupting this *damage loop*. During the last decade, major advances in the field of podocyte biology have been achieved through studying the structure of their foot processes. Numerous new slit diaphragm and cytoskeletal proteins have been discovered, and treatment strategies targeting these proteins in vitro have shown promising results. However, restoring levels of single podocyte structure proteins on their own in kidney disease will probably not be effective; the architecture of the podocyte foot processes and their slit diaphragms is particularly complicated and involves hundreds of different proteins, and their levels and interactions are delicately balanced. A gain- or loss of function in one of these proteins might even result in disruption of podocyte architecture and irreversible glomerular damage.

Chapter 6 - VEGF splicing variants in the kidney

Overall, **chapter 5** highlights the importance of VEGF in the cross-talk between podocytes and endothelial cells in the healthy glomerulus. Remarkably, it still remains an enigma how this cross-talk is established; somehow, VEGF manages to cross the glomerular basement membrane in the opposite direction of the filtrate flow, and reaches the endothelial cells. Clues might lie within the composition of the renal splicing pattern

of VEGF; the VEGF mRNA can be spliced alternatively, resulting in at least 8 different isoforms. Some of these isoforms are short and can diffuse freely; others possess heparin binding sites and are stored and transported in extracellular matrix until release.[8] To gain more insight on VEGF crosstalk in the glomerulus in kidney pathology, **chapter 6** focuses on renal VEGF splicing patterns in health and disease.

First, mRNA splicing patterns of pro-angiogenic isoforms of VEGF in glomeruli from human and murine control subjects were determined with capillary electrophoresis. Strikingly, the splicing patterns were revealed to be species-specific; in the control human kidney samples, the short and freely diffusible VEGF 121 was the dominant isoform, whereas the longer and more potent VEGF 164 was the dominant isoform measured in the mouse kidney samples. This suggests different mechanisms of VEGF transport in human and murine kidneys, and brings to question if studies on glomerular VEGF in murine models can be extrapolated to the human situation.

Subsequently, VEGF splicing patterns during endothelial dysfunction and under inflammatory conditions were measured in kidney samples from patients with diabetic nephropathy and from patients with acute rejection following kidney transplantation, respectively. In addition, kidney samples from mice with lupus nephritis and mice with diabetes mellitus were studied. The pattern of renal VEGF-A splice variants was unchanged in diabetic nephropathy and lupus nephritis and was stable throughout disease progression in acute transplant rejection and diabetic nephropathy; these results suggest renal VEGF-A splicing stability during kidney disease. This could, on the one hand, imply that renal splicing is not affected by inflammatory or metabolic kidney damage. On the other hand, these results could show inability of the podocyte to change its splicing pattern towards a more favourable one for survival during challenging circumstances.

GENERAL DISCUSSION

The work described in this thesis underlines the complexity of the aetiology of preeclampsia. In the placenta, loss of cytoprotection, increased coagulation and increased inflammation may give rise to placental dysfunction and subsequent production of anti-angiogenic factors, as strengthened in **chapters 2 and 3**. However, the predisposing factors for preeclampsia are so heterogeneous, that preeclampsia and its placental dysfunction might merely

reflect a phenotype resulting from various syndromes or diseases, composed of more confined aetiologies. Below, this hypothesis is explored and the results from this thesis are considered in this setting.

THE AETIOLOGY OF PLACENTAL DYSFUNCTION IN PREECLAMPSIA: A SPECTRUM RANGING FROM COAGULATION TO IMMUNITY?

Although an imbalanced inflammatory environment in the placenta during early development has been proposed as the primary mechanism for placental dysfunction in preeclampsia,[9] **chapter 2** of this thesis mainly provides support for inherited thrombophilia as a genetic contributor to preeclampsia. These results suggest that the immune component of the aetiology of preeclampsia is probably composed of acquired risk factors, which offers perspective for the targeting of these risk factors in prevention of the syndrome. These observations gave rise to a hypothesis where preeclampsia comprises of a spectrum of pregnancy complications with the placenta as the central role player, but with different underlying aetiologies of the placental dysfunction, ranging from inherited thrombophilia to extreme immune dysregulation (Figure 1). This is of particular interest, because treatment strategies targeting downstream effects of preeclampsia have not been effective so far; targeting processes upstream from endothelial dysfunction might be a more effective approach. Each of these possible factors contributing to placental dysfunction and suggestions for their individual approach will be discussed below.

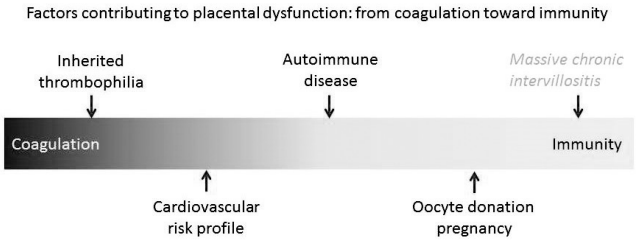

Figure 1 Factors contributing to placental dysfunction: from coagulation towards immunity

It is hypothesized that the underlying aetiologies of placental dysfunction in preeclampsia lie within a spectrum, ranging from coagulation towards immunity

Inherited thrombophilia

A clear, distinct contributor to the development of placental dysfunction in preeclampsia would appear to be inherited thrombophilia; e.g. with mutations in genes encoding for factor V, prothrombin and SERPINE1, as illustrated in **chapter 2**. Women with these mutations have increased activation of the coagulation cascade, putting them at risk for developing deep venous thrombosis and preeclampsia.[10] A rationale for this might lie within the communication between embryonic cells and the mother's coagulation system through thrombin, thrombomodulin and the PAR-1 receptor;[11] excessive coagulation and disturbed PAR-1 signalling could subsequently lead to impaired trophoblast function and subsequent placental dysfunction, as illustrated by our own results in **chapter 3.**

However, the thrombophilic mutations described in **chapter 2** have a low penetrance regarding the preeclampsia phenotype; a 'second hit' and other risk factors clearly are essential, perhaps from the lifestyle category discussed below.[12] Although these variants remained associated with preeclampsia after meta-analyses, others still question the association between these mutations and preeclampsia completely, mentioning possible publication bias favouring small studies reporting high relative risk ratios.[13] Nevertheless, inhibiting the coagulation and complement systems with heparins in women with inherited thrombophilia after their first adverse pregnancy outcome decreases the risk of foetal growth restriction, implying a positive effect on the placenta.[12, 14] All in all, the contribution of inherited thrombophilias to the aetiology of preeclampsia seems present but not substantial without other contributing factors. Other, more effective ways of targeting the coagulation system have to be investigated before genetic screening for and treatment of inherited thrombophilia becomes effective for the prevention of preeclampsia in pregnant women.

Cardiovascular risk factors

This brings us to the second etiological pathway, which is particularly exciting, because it might provide us with the most accessible handles for preventing preeclampsia. This second pathway consists of adverse cardiovascular circumstances predisposing for preeclampsia. The greatest contributors in this category are obesity, metabolic syndrome, hypertension, and diabetes,[15, 16] and the share of these contributors is probably only going

to grow in the next decade due to the obesity-epidemic. In Europe and the United States of America, more than half of the population is overweight today.[17] Interestingly, the incidence of preeclampsia has grown along with the obesity incidence.[18]

Obesity and its comorbidities are associated with dysregulation of both coagulation and inflammation, so this group lies more towards the middle of the spectrum. All these risk factors namely contribute to endothelial dysfunction prior to pregnancy, reducing the endothelial reserve to cope with challenging circumstances such as pregnancy. For example, women with chronic hypertension prior to pregnancy develop preeclampsia more often than normotensive pregnant women, but when they develop preeclampsia, the sFlt-1/ placenta growth factor ratio is smaller than in normotensive subjects, indicating increased sensitivity to small changes in this ratio.[19]

More in-depth, obesity, metabolic syndrome and diabetes cause endothelial dysfunction through various pathways, including hyperlipidaemia, increased inflammation and insulin resistance.[20] Interestingly, **chapter 2** of this thesis strengthened this hypothesis by underlining the link between lipoprotein lipase mutations and preeclampsia. The contribution of this predisposing mechanism is illustrated further illustrated by **chapter 4** from this thesis, where models of diabetes and metabolic syndrome show increases in glomerular thrombomodulin expression similar to those seen in preeclampsia.

Adverse cardiovascular circumstances could even contribute to the pathophysiology of preeclampsia during the placental development stage; they have been proposed to enhance vascular dysfunction of the decidual arteries, possibly through increased inflammation and disrupted remodeling,[21] which could contribute to diminished placental blood supply. Also, interference with the normal coagulation-trophoblast interaction through PAR-1, as mentioned above, could contribute to impaired trophoblast invasion. This thought is strengthened by the results from **chapter 3** from this thesis, where the extent of placental thrombomodulin loss was correlated to maternal BMI and blood pressure, and to placental dysfunction.

The great impact of these metabolic disturbances on the endothelium even prior to pregnancy might provide an explanation for the modest effect of treatment targeting downstream effects of coagulation and inflammatory pathways, such as aspirin and heparin. Treatment strategies targeting the dysfunctional endothelium itself and diminishing the endothelial activation

might have a more potent effect. Perhaps activating PAR-1 signalling through thrombomodulin to enhance anti-inflammatory and anti-coagulative signalling, as in more severe examples of endothelial dysfunction, could be of use here.

Autoimmune disease and preeclampsia

Slightly further towards the immunological end of the spectrum we find preeclampsia in the setting of autoimmune diseases. Some autoimmune diseases which are associated with renal involvement themselves are associated with a particular high risk of preeclampsia, for example, the risk of preeclampsia in systemic lupus erythematosus can increase to up to 40 percent.[22] However, the presence of autoantibodies, in particular antiphospholipid antibodies, independent of disease activity is associated with preeclampsia.[23, 24] The imbalanced immune system and in particular the T-cells have been proposed to be involved in the development of preeclampsia in the setting of autoimmune disease.[22] Nevertheless, heterogeneity in the extent of the autoimmune disease prior to pregnancy appears to be of importance as well when calculating the risk for obstetric complications, pointing towards the 'endothelial reserve' hypothesis described above.[22] So far, maximizing care and minimizing symptoms prior to pregnancy still appear to be the most feasible options for preventing obstetric complications in autoimmune disease.

Oocyte donation

A distinct form placental dysfunction occurs with preeclampsia in the setting of an oocyte donation pregnancy. Oocyte donation is a technique that involves harvesting of oocytes from a donor, subsequent fertilization of the ovum with sperm in vitro, and transfer of the embryo in the recipient mother's uterus. If implantation succeeds, logically, this results in a foetus that is completely allogeneic to the mother, creating a challenging environment for the mother's immune system, comparable with the setting of organ transplantation. This allogeneic foetus appears to be the main 'hit' for the development of preeclampsia after oocyte donation. Further, women receiving oocyte donation are generally older than women with an autologous pregnancy, putting them at an increased risk of developing

pregnancy complications and experiencing 'second hits' such as diabetes or hypertension.[25]

The immunomodulating mechanisms of early pregnancy described in the introductory chapter of this thesis do not suffice in oocyte donation pregnancy, and characteristic changes can be found in the oocyte donation placenta, such as villitis of unknown aetiology and chronic deciduitis.[26] These changes are much more prominent than those in the preeclamptic placenta from naturally conceived pregnancies; for instance, in **chapter 3** of this thesis, no differences were found in placenta infiltrates between cases and control subjects, despite loss of the inflammation-modulating thrombomodulin. Further, direct evidence of differences in regulation of the innate and adaptive immune systems between naturally conceived and oocyte donation pregnancies reveals reduced regulation of the complement system and increased T-cell activation in oocyte donation pregnancies.[27, 28]

These changes in immunoreactivity are likely contributors to the development of placental dysfunction in preeclampsia; as described in the introductory chapter, delicate balances between pro- and anti-inflammatory subtypes of immune cells have to be established in the developing placenta to facilitate assistance for the trophoblast cells during invasion and remodelling of the placenta. Disturbances in the composition of these macrophage, NK-cell or T-cell populations are associated with impaired placentation. So far, mainly term placentas from women with oocyte donation pregnancies have been studied for their immune population compositions. In-depth studying of the early changes in placental immune cell composition during preeclampsia in oocyte donation pregnancies might provide therapeutic targets for prevention of immune-mediated forms of preeclampsia.

Massive chronic intervillositis

An extreme variant of placental dysfunction due to immune dysregulation is massive chronic intervillositis of the placenta. Massive chronic intervillositis is characterized by a wide-spread intervillous infiltrate composed of T-cells and macrophages, somewhat resembling a form of chronic rejection, however, the macrophages make up over 80% of the infiltrate.[29] Malaria infection during pregnancy can be associated with a similar placental infiltrate, but to date, no infectious agent has been identified in massive chronic intervillositis.[30] Chronic intervillositis has been reported to be associated

with foetal growth impairment, intra-uterine foetal death, and, unfortunately, has a high recurrence rate.[31] Interestingly, the syndrome is associated with antiphospholipid syndrome, suggestive of an aetiology involving autoimmunity.[32]

To date, it remains a mystery what attracts the macrophages in massive chronic intervillositis. In preeclampsia, excessive amount of M1-type, pro-inflammatory macrophages are attracted to the placenta in reaction to oxidative stress.[33] In massive chronic intervillositis, an adverse event such as large decidual artery infarction or placental dysfunction during early stages of pregnancy might provoke a similar, but enhanced reaction. This severe placental syndrome does not give rise to maternal symptoms and therefore does not fit into the spectrum of preeclampsia completely, but studying an extreme variant of immune dysregulation in the placenta might provide new, confined mechanisms regarding the regulation of inflammation in the placenta that can be extrapolated to the setting of preeclampsia, and vice versa.

GLOMERULAR VEGF CROSS-TALK

All in all, different pathways appear to contribute to the development of placental dysfunction in preeclampsia, ranging from dysregulation of coagulative to inflammatory processes. So far, kidney involvement in preeclampsia appears to have a more confined aetiology, and appears to be mainly caused by the angiogenic imbalance. This is supported by findings in animal models of anti-angiogenic circumstances, which show glomerular changes similar to preeclampsia. Further, removal of sFlt-1 from the circulation in women with preeclampsia has shown to reduce proteinuria significantly in a small trial recently.[34] Also, other hallmarks of preeclamptic nephropathy, such as complement and fibrin deposition,[35] appear to lie downstream of the angiogenic imbalance: both appear after treatment with anti-angiogenic agents.[35, 36]

Below, the impact of an angiogenic imbalance on the kidney and key points for future research are discussed.

Future: interference with glomerular crosstalk

Anti-angiogenic factors interfere with the delicate balance in the cross-talk between podocytes and endothelium, which leads to a damage loop, as supported in **chapters 4, 5 and 6**. Further research into glomerular VEGF cross-talk should comprise the exact role and transportation of sFlt-1 and VEGF in the kidney. As described in chapter 6, the freely diffusible VEGF121 is the dominant VEGF isoform in the human kidney. This isoform lacks heparan binding sites and could therefore, theoretically, freely pass the glomerular basement membrane to reach the endothelial cells. However, the filtration pressure over the glomerular basement membrane approaches 20 mmHg and subsequently results in shear stress with pressures reaching 8 Pa on the podocytes near the basement membrane.[37] The enigma remains how podocytal VEGF 121 could reach the glomerular endothelial cells in the opposite direction of these forces; it would appear more logical for VEGF121 to be washed away with the urine. Perhaps VEGF 121 is involved in local or autocrine VEGF signalling between podocytes, and the longer isoform VEGF 165 is transported over the glomerular basement membrane through binding to heparans. Still, in the setting of preeclampsia, this seems illogical: the glomerular filtration rate diminishes in preeclampsia and glomerular blood flow decreases, presumably leading to decreased filtration pressure and shear stress on the podocytes, logically making it easier for VEGF to pool and reach the endothelial cells.[38]

Obviously, the excessive sFlt-1 levels in preeclampsia prevent the podocytal VEGF from binding to its receptors on the endothelium and podocytes; sFlt-1 can freely pass the glomerular basement membrane and can be measured in urine from women with preeclampsia.[39] However, results from thesis **chapter 4**, together with previous results from other groups,[40] show increases of renal VEGF expression under anti-angiogenic circumstances. This raises the question if this increase in angiogenic factors would not be enough to compensate for the increased sFlt-1 levels. Of course, the anti-angiogenic imbalance of preeclampsia leads to a distinct renal phenotype, but a recent study shed new light on the role of sFlt-1 in kidney disease. Jin et al revealed that podocytes possess an sFlt-1 receptor which regulates their actin cytoskeleton arrangement.[41] Further, this sFlt-1 receptor seems to be present on pericytes in the rest of the vasculature as well. Possibly, the increased

sFlt-1 levels in preeclampsia act on these pericyte receptors and promote the hypertensive phenotype this way.
Interfering with VEGF cross-talk in the glomerular damage loop may not be the only promising option for targeting preeclampsia; recent studies have revealed promising results by interfering with glomerular cross-talk on the level of the endothelium. The endothelin pathway appears to be a promising target: blocking endothelin signalling in a mouse model of diabetic nephropathy ameliorated proteinuria.[42] Endothelial endothelin expression is increased in preeclamptic women as well, making it appear as a promising target.[43] However, endothelin expression is a downstream effect of endothelial activation, and targeting the endothelial activation of preeclampsia as a whole would probably more effective and subtle, as endothelin is an important vasoconstrictor and blocking it completely will not be possible for humans. An inhibitor of endothelial activation is thrombomodulin; as described in **chapter 4**, levels of glomerular thrombomodulin increase as a counter-mechanism under anti-angiogenic circumstances, but this increase is not sufficient. Soluble thrombomodulin administration appears to be a promising way to restoring glomerular function in mouse models of inflammatory kidney disease;[44] perhaps treatment with soluble thrombomodulin could have a beneficial effect on glomerular endothelium, and subsequently on glomerular cross talk and endothelin expression, under anti-angiogenic conditions as well.

Irreversible damage after preeclampsia?

After delivery of the placenta, levels of anti-angiogenic factors in serum return to normal and proteinuria resolves in most women with preeclampsia. However, persistent podocyte loss after resolution of preeclampsia has been described,[45] and women who have had preeclampsia remain at an increased risk for developing chronic kidney disease.[46] The ongoing and increasing damage even after resolution of the anti-angiogenic environment suggest an irreversible and continuing process in the glomerulus after preeclamptic nephropathy.
Recently, Kriz et al proposed an interesting paper on a hypothesis where loss of several podocytes leads to increased mechanical stress on other podocytes, leading to irreversible podocyte damage and loss.[37] In contrast to the glomerular endothelium, which can replicate and expand easily,

and the glomerular basement membrane, which has never been reported to break under physiologically possible ranges of pressure, the podocytes are particularly sensitive to increased filtration through the glomerulus. Podocytes have a limited capacity to expand with increasing GBM areas due to the definite width of the slit diaphragm, and cannot be replaced effectively when they are damaged. Loss of podocytes, as in preeclampsia, leads to an increased working load for the remaining podocytes, and subsequent increased mechanical challenges. According to Kriz, this leads, in the long term, to glomerulosclerosis.
In patients who experienced preeclampsia, this process might be accelerated by the unfavourable cardiovascular circumstances associated with preeclampsia; diabetes or hypertension increase glomerular stress and podocyte working load even further. Therefore, physicians should be cautious when treating a patient with cardiovascular problems and preeclampsia in their patient history; these women might be at an increased risk for developing renal failure as well.[46]

Preeclampsia: a 'model' disease?

Kidney disease in most women with preeclampsia runs relatively mild. However, preeclamptic nephropathy is of particular interest for the rest of the field of kidney research; preeclampsia can be viewed as an extreme example of the disruption of glomerular VEGF crosstalk during a short period of time. Observing the changes and mechanisms involved in restoring this cross-talk mild provide suggestions for research into other forms of proteinuric kidney disease. Milder, but chronic forms of angiogenic imbalance play a role in for example diabetic nephropathy and FSGS, which is also associated with disrupted TGF-beta signaling.[47, 48] Recently, different polymorphisms of the VEGF gene have even been reported to be associated with hypertension-related chronic kidney disease.[49-51] Since VEGF cross-talk appears to act differently in the human and murine kidney, as suggested in **chapter 6**, patients with preeclampsia might be the best 'in vivo' example of an anti-angiogenic state. However, this might be unpractical because biopsy specimen from patients with preeclampsia are rare or even non-existent, since kidney biopsy is a relatively hazardous procedure, especially during pregnancy and the cause of kidney disease in preeclampsia usually is evident, so the need for a biopsy is often absent.

FUTURE PERSPECTIVES

In conclusion, preeclampsia appears to be a two-stage disease, starting with disrupted placental formation through both vascular and immunological challenges early during pregnancy, and resulting in maternal systemic involvement, with the vascular system and the kidney as the first organs targeted. However, the reverse is also true, as noted in this discussion; pre-existent vascular damage and kidney disease prior to pregnancy also put women at risk for developing preeclampsia, probably by decreased reserves for coping with the increased demands of pregnancy. So, cause and consequence appear to be intertwined in the aetiology of preeclampsia, resulting in a complex, multifactorial pathogenesis. This can also possibly explain why treating single targets from the immune or coagulation system at once, during later stages of the disease, does not resolve the symptoms of preeclampsia.

In the discussion of this thesis, a new way of unravelling this multifactorial aetiology has been proposed; the different risk factors for preeclampsia, such as thrombophilia, cardiovascular risk factors, oocyte donation and autoimmune disease might all result in different aetiologies of the same syndrome, requiring an individual approach. The role of the placenta, and its early changes, might differ between these different etiological pathways leading up to preeclampsia. The late changes in the placenta in preeclampsia have been studied extensively, but the early stages of the placenta in preeclampsia in human patients have remained underexposed. However, such material is, of course, hard to obtain; placenta biopsies cannot be performed for research purposes, and with materials from first-trimester miscarriages it remains unknown whether the patient would have developed preeclampsia. A feasible alternative for detecting changes during early stages of pregnancy is proteomics analysis. With proteomics, all proteins expressed in a tissue or blood sample are measured and their levels can be compared with other samples.[52] This “drag-net” like method might reveal new serum markers that reflect placental changes in certain types of patients; for preeclampsia in general, several new role players such as complement factor C1 and clusterin, involved in apoptosis, have been discovered recently with proteomics.[53, 54] New biomarkers might allow us to distinguish between primary placenta-mediated preeclampsia, with major involvement of immune dysregulation, from preeclampsia with a major role for decreased vascular reserve. This

might also set the stage from patient-tailored therapy; some patients might benefit from anti-inflammatory or anti-coagulative drugs targeting the systemic response, and others might benefit from immunomodulating drugs early during pregnancy, during placental formation.

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