On renal pathophysiology in preeclampsia

Penning, M.E.

VII Summary and general discussion

Summary

Preeclampsia is a complication of pregnancy which can suddenly change from a relatively mild phenotype into a life-threatening situation. One of the organs that is always involved during preeclampsia is the kidney. The placenta plays an important role in the renal pathophysiology of preeclampsia. The placenta produces excessive amounts of anti-angiogenic factors which are associated with systemic endothelial dysfunction. Although the underlying mechanisms of renal injury during preeclampsia remain unclear, women with preeclampsia have an increased risk of developing renal disease later in life. This observation suggests that preeclampsia 'marks' the mother—putatively in combination with pre-existent conditions—which might contribute to serious sequel throughout her life.

The widespread endothelial dysfunction in preeclampsia is believed to be due to increased serum levels of anti-angiogenic factors—in particular sFlt-1—produced by the placenta.[1] The studies described in CHAPTER 2 were focused on the mechanisms of sFlt-1 production within the placenta, and the systemic spreading of this anti-angiogenic factor. During both preeclampsia and uncomplicated pregnancy, the placenta is the principal source of sFlt-1. The outermost layer of the placenta, the syncytiotrophoblast, forms 'knots' that contain the anti-angiogenic protein sFlt-1, particularly during preeclampsia. Previous research has shown that these knots—called syncytial aggregates after detachment from the placenta—account for approximately 25% of all sFlt-1 protein in the maternal circulation.[2] In this thesis, the spread of these syncytial aggregates was further investigated. We showed that—within the placenta—syncytial knots are indeed the primary production site of sFlt-1, which is significantly higher in placentas from patients with preeclampsia. These knots detach from the placenta, and the systemic spread of the syncytial aggregates was confirmed by the presence of hCG-positive multinucleate aggregates in the lungs of pregnant women. We showed that preeclampsia is associated with higher numbers of syncytial aggregates in the maternal lung. In lung samples obtained from women who were carrying a male fetus co-localization of hCG and the Y-chromosome was observed, which strongly supports the idea that these aggregates are of fetal origin. Importantly, we observed that women with preeclampsia show increased sFlt-1 expression within these aggregates, as compared to the non-preeclamptic pregnant control subjects. It may be speculated that these aggregates influence the development of local immune tolerance in maternal organs early in pregnancy. Later in pregnancy, the sFlt-1 produced by these aggregates may cause endothelial injury. The presence and persistence of fetal cells in the maternal circulation and organs may also have both short- and long-term consequences for postpartum maternal health. On short-term, the sFlt-1-loaded syncytial aggregates may undergo further disaggregation, forming smaller particles. Via the release of these smaller particles into the maternal circulation, it is not unlikely that these sFlt-1-loaded smaller particles contribute to endothelial dysfunction in maternal organs other than the lungs. On long-term, the fetal cells may result in chimerism, as they can be retained in the maternal blood and organs for decades after delivery.[3] These cells—having stem cell-like properties—might affect maternal health for years after pregnancy, but it is currently unknown whether they cause disease or might have beneficial effects, or both.[4, 5]
Within the kidney, endothelial dysfunction can be caused by an imbalance between anti- and pro-angiogenic factors. In particular, vascular endothelial growth factor-A (VEGF-A)-signaling—necessary for the maintenance of the glomerular filtration barrier—seems to be affected during preeclampsia. Putative mechanisms playing a role in renal injury during preeclampsia were investigated in the studies described in CHAPTERS 3 and 4. Previous studies have shown that injury to the fenestrated endothelium within the glomerulus can lead to complement activation.[6] Combining this observation

with the notion that preeclampsia is characterized by complement dysregulation—shown in placentas[7-9] and serum[10]—we explored complement activation in the preeclamptic kidney in the studies described in chapter 3.

In the studies described in chapter 3 we investigated renal autopsy tissues from women who died due to preeclampsia, as well as control subjects, for the presence of complement components. Preeclampsia was significantly associated with the presence of C4d—a stable marker of complement activation—and markers of classical pathway activation. To explore whether complement activation during preeclampsia in the kidney is caused by antibody-mediated injury, we performed immunofluorescence stainings on the renal autopsy tissues mentioned, for the presence of immunoglobulins. Immune deposits of the IgM-subtype were significantly more frequently observed in kidneys of women with preeclampsia, whereas IgG deposits were sporadically observed in all study groups, and IgA was never observed. It is not possible to investigate the causative role of sFlt-1 in the activation of the complement system in the kidney in vivo in humans. Therefore, a sFlt-1-induced-mouse model of preeclampsia was used. Mice injected with sFlt-1 show increased deposition of C4 in the kidneys as compared to control mice. These findings suggest that angiogenic imbalance may play an important role in activation of the complement system in the kidney during preeclampsia. The observations described in this chapter raise the question: what causes the presence of the IgM-subtype in glomeruli of patients with preeclampsia? Although glomerular IgM deposition is observed in a variety of renal diseases and its role remains elusive, the presence of this subtype might have several explanations. Firstly, the presence of the IgM-subtype could reflect the binding of IgM antibodies to endothelium injured by sFlt-1. Natural IgM-antibodies play a major role in the clearance of damaged cells,[11, 12] and they can bind to both hypoxic[13] and apoptotic cells[14, 15] through intracellular antigens

that become externalized under these conditions. Secondly, IgM is a large molecule, and due to its polymeric nature, it easily binds non-specifically to endothelium.[16] Finally, although we did not observe other immunoglobulin subtypes, it may be hypothesized that the presence of IgM might be caused by auto-antibodies.[17] Altogether, the studies in this chapter show that preeclampsia is strongly associated with activation of the classical pathway of the complement system in the kidney. These findings suggest that complement activation might amplify local inflammation, and could contribute to the renal injury in preeclampsia. Our findings further support the concept of complement inhibition as a therapeutic tool, targeting both the renal and placental manifestations of preeclampsia.

The extent of renal injury during preeclampsia was investigated in the studies described in chapter 4. Preeclampsia is characterized by loss of podocytes into the urine. Therefore, this chapter was devoted to quantification of the numbers of podocytes within the glomeruli of patients with preeclampsia and of control subjects, using the same renal autopsy tissues as in chapter 3. The numbers of glomerular podocytes did not differ significantly between the preeclamptic and control groups. Podocyte injury and loss are often associated with increased parietal epithelial cell activation. Therefore, we evaluated parietal epithelial cell activation in the setting of preeclampsia. Women with preeclampsia showed significantly higher numbers of activated parietal epithelial cells on a podocyte location. It remains to be investigated whether activation and proliferation of parietal epithelial cells compensate for ongoing podocyte injury and loss, or whether these cells contribute to glomerular injury, or both. The findings described in this chapter may provide new insights into the renal complications of women with preeclampsia later in life. Parietal epithelial cell activation is associated with focal and segmental glomerulosclerosis (FSGS),[18] and women with preeclampsia have a higher risk of

developing FSGS later in life.[19] As further discussed below, we hypothesize that the higher numbers of activated parietal epithelial cells in the preeclamptic kidney as described in this chapter, may play an important role in the pathogenesis of the development of FSGS later in life.

It is evident from the studies described in chapters 3 and 4, that the kidney—and in particular, the glomerulus—is targeted during preeclampsia. An important cause of this, as mentioned earlier, is believed to be the angiogenic imbalance, in which an excess of particularly sFlt-1 prevents VEGF-A from adequately maintaining the renal endothelium. As a VEGF-A-signaling pathway is responsible for cross-talk between endothelial cells and podocytes, it is believed that both endothelial cells and podocytes are injured in situations of angiogenic imbalance. Injured podocytes may detach from the glomerular basement membrane. After detachment from the glomerular basement membrane, podocytes are shed into the urine. Podocyturia during preeclampsia was further investigated in the studies described in CHAPTER 5. In this chapter, we explored the potential of using quantitative polymerase chain reaction (qPCR) as a means to detect the mRNA levels of podocyte-specific molecules in urine samples of women with preeclampsia and of control subjects. Women with preeclampsia showed significantly elevated urinary mRNA levels of nephrin, podocin, and VEGF as compared to normotensive pregnant control subjects, and non-pregnant control subjects. In addition, significantly elevated levels of nephrin mRNA were detected in urine of women with preeclampsia as compared to that of women with gestational hypertension. We found mRNA encoding for podocyte-specific molecules in urine of non-pregnant women, normotensive pregnant women, and women with gestational hypertension, which indicates that during all these conditions, there is a (physiological) loss of podocytes in urine. It may be speculated that normal pregnancy, gestational hypertension, and preeclampsia are a continuous spectrum of (mild) endothelial

dysfunction, contributing to podocyturia. The results from the studies described in this chapter demonstrate that qPCR is a highly promising, non-invasive method for quantifying podocyturia in patients with preeclampsia, but it still needs to be assessed whether measuring podocyturia is a more reliable marker than conventional biomarkers are. Importantly, a very recent study showed that podocyturia precedes proteinuria and the clinical features of preeclampsia,[20] which is discussed in detail on page 168 of this chapter.

To identify possible first-trimester biomarkers in preeclampsia, the work described in CHAPTER 6 focused on the presence of metabolites in early-pregnancy urine samples, and their potential role in predicting preeclampsia. The set of metabolites that reflects the organism under a particular set of conditions, is called the *metabolome*.[21] The metabolome is the product of environmental and genetic conditions, and provides a new logical framework to elucidate disease etiology. In the studies described in chapter 6, first trimester urine samples were analyzed using nuclear magnetic resonance spectroscopy. The concentrations of 36 metabolites were compared in a nested case-control study of 73 women who developed preeclampsia later during pregnancy and 138 pregnant control subjects derived from a prospective cohort study consisting of 33.602 singleton pregnancies.[22] Although ethnicity appeared to be a strong confounding factor, we identified a first trimester urinary metabolomics signature within the two largest ethnical subgroups—Caucasians and Blacks—for patients developing preeclampsia later in pregnancy. The significantly different urinary metabolomics signature in the first trimester of pregnancy in women destined to develop preeclampsia, potentially holds the promise of a screening test, and offers insight into the pathogenesis of preeclampsia.

General Discussion

ON RENAL PATHOPHYSIOLOGY IN PREECLAMPSIA

Because the kidney is always involved in preeclampsia, the causes and consequences of kidney injury, both on the short- and long term, will be discussed below.

CAUSES AND CONSEQUENCES OF ENDOTHELIAL INJURY

As mentioned earlier, endothelial injury is considered to play an essential role in the pathogenesis of preeclampsia. A significant part of this injury is thought to arise by the excessive amounts of the anti-angiogenic factor sFlt-1, which is produced by the placenta during pregnancy. However, not all pregnant women with high sFlt-1 serum concentrations develop preeclampsia. The overlap of sFlt-1 concentrations between women with preeclampsia and women with uncomplicated pregnancies illustrates this phenomenon.[1] Therefore, the rise in sFlt-1 can not solely be held accountable for the endothelial injury observed during preeclampsia. It remains debatable why some patients are so sensitive to high concentrations of sFlt-1, whereas other patients abide the high concentrations of sFlt-1 very well. sFlt-1 binds to VEGF-A, which leads to low concentrations of bio-active VEGF-A.[23, 24] Thereby, the physiological functions of VEGF-A are impaired, including proliferation, angiogenesis, lymphogenesis, inducing endothelial cell permeability, and vasodilation.[23, 24] Perhaps, pre-existent endothelial injury increase the vulnerability for a rise in sFlt-1. It is known that conditions associated with endothelial injury predispose to preeclampsia, such as diabetes mellitus, obesity, and hypertension.[25] It may be that women with such pre-existent endothelial injury are more vulnerable to the stress imposed on the endothelium during pregnancy.[26-28] Thus, the rise in sFlt-1 which occurs during preeclamptic pregnancy might aggravate pre-existent endothelial injury. Furthermore, other anti-angiogenic factors, such as soluble Endoglin,[29] have been described to play an important role in the endothelial injury during preeclampsia. These observations support the hypothesis that endothelial injury during preeclampsia is a multifactorial condition. Taken together, there is an overlap between the concentrations of anti-angiogenic factors in healthy pregnant women and women with preeclampsia. This phenomenon could be explained by the assumption that healthy pregnant women are not as sensitive to high concentrations of anti-angiogenic factors, or might not have pre-existent endothelial injury, or both. Rising levels of anti-angiogenic factors alone may not be sufficient to cause systemic endothelial injury.

Preeclampsia is associated with certain genetic variants.[30] These genetic variants concern genes associated with the coagulation, fibrinolysis, immunological, and renin-angiotensin-aldosterone systems. In addition, these genetic variants also seem to concern genes involved in cardiovascular disease and its risk factors, such as diabetes mellitus, obesity, and hypertension.[30] Illustratively, women with preeclampsia have an 8-fold higher risk of death from cardiovascular causes later in life than women who have had an uncomplicated pregnancy.[31] However, not all women with preeclampsia do develop cardiovascular disease later in life.[32, 33] The question that remains is, whether preeclampsia is the cause of remaining endothelial injury, causing cardiovascular disease later in life, or that the genetic make-up and other risk factors of these women result in pre-existent endothelial injury and predispose to both preeclampsia and cardiovascular disease.

The different genetic predispositions associated with preeclampsia could also be the reason for distinct metabolic profiles in women with preeclampsia, compared to pregnant control subjects. In a recent study, a metabolomics signature in serum was described to be specific for preeclampsia.[34] In the results described in chapter 6, we observed that the levels of specific metabolites differ in first-

trimester urine samples between patients who develop preeclampsia and control subjects, and we found that ethnic background was a confounding factor. These findings may provide a viable method for predicting preeclampsia early, even before symptoms develop, and may provide key insight into the disease's pathogenesis. It may be, that the factors playing a role in early pregnancy could also contribute to the pre-existent endothelial injury, and subsequent vulnerability for sFlt-1 in preeclampsia.

Within the murine preeclamptic kidney, endothelial injury through high sFlt-1 levels manifests as endotheliosis (swelling of endothelial cells).[35] However, there is increasing evidence that complement activation also contributes to the kidney injury in preeclampsia. The results described in chapter 3 are the first observations to suggest a putative role that injured endothelium itself might play in initiating activation of the complement system during preeclampsia. In particular, the presence of complement deposits in a sFlt-1-induced-mouse model of preeclampsia, strongly supports the hypothesis that sFlt-1 induced endothelial injury is—at least in part—responsible for activation of the complement system. Activation of the complement system can be the result of binding of auto-antibodies.[36, 37] In the kidney specifically, complement activation could result from binding of auto-antibodies to glomerular structures, or by glomerular deposition of circulating immune complexes.[6] In preeclampsia, immune complex deposition and subsequent complement activation could, for instance, result from binding of angiotensin II type 1 receptor agonistic antibodies (AT1-AA). These antibodies are frequently observed in women with preeclampsia,[17] and in a murine model the presence of these antibodies resulted in glomerular complement activation.[38] However, not all women with preeclampsia have circulating antibodies against the angiotensin II type I receptor. In the work described in chapter 3 of this thesis, glomerular complement activation was observed in all studied women with preeclampsia. Although the number of patients studied was relatively small, this finding suggests that

other mechanisms than binding of auto-antibodies might also be responsible for activation of the complement system. Indeed, another putative cause for complement activation is the interplay between the complement system and dysregulation of pro- and anti-angiogenic factors resulting in angiogenic imbalance. Within the kidney there is evidence that complement activation is a consequence of angiogenic imbalance. The high concentrations of circulating sFlt-1 bind to VEGF-A, which leads to low concentrations of bio-active VEGF-A.[23, 24] In vivo cell experiments showed that on the podocytes' surface, low concentrations of VEGF-A lead to reduced concentrations of the important complement regulator, factor H.[39] Dysfunction of factor H is also associated with atypical Hemolytic Uremic Syndrome (aHUS).[40] Since aHUS and preeclampsia show overlapping histopathologic renal lesions—such as double contours of the glomerular basement membrane and signs of thrombotic microangiopathy—the underlying pathogenic mechanisms of these conditions may share the same pathways. In aHUS, the presence of mutations in factor H is associated with the deposition of complement products on the surface of platelets, resulting in a prothrombotic state.[41] The other way around, complement may be activated on the membrane of activated platelets.[42] Thus, these findings may suggest that complement dysregulation during preeclampsia, in particular regarding factor H, is linked to conditions associated with high incidence of thrombosis, which indeed seems to be the case.[43] This multifaceted vicious cycle may aggravate the kidney injury during preeclampsia. In following, there are several questions that need to be answered. Is activation of the complement system a cause or a consequence of endothelial injury? Is activation of the complement system within glomeruli of patients with preeclampsia a result of sFlt-1 induced endothelial injury? Or is the complement system activated through autoantibodies directed against the angiotensin II type I receptor, thereby causing endothelial injury? And does this endothelial injury then cause activation of the complement system, and thereby activation of the coagulation system?

PODOCYTURIA: PREDICTION AND A GLIMPSE INTO THE FUTURE

Endothelial injury is thought to give rise to the clinical manifestations of preeclampsia, including hypertension and kidney injury.[35] Clinically, this kidney injury results in worsening of kidney function and loss of proteins in the urine: proteinuria. Within the relatively short period of preeclamptic pregnancy, the glomerular filtration barrier is severely injured, which leads to leakage of proteins. Interestingly, Yu et al.[44] demonstrated that in rat models of puromycin aminonucleoside-induced nephrosis, mesangioproliferative nephropathy, and hypertensive nephropathy, proteinuria is present during both active and chronic phases of glomerular injury, whereas podocyturia is confined to active, ongoing glomerular damage only. Based on these findings, podocyturia seems to be a good marker of ongoing glomerular damage.

In the work described in chapter 5 of this thesis, increased podocyte specific mRNA levels were observed in urine of women with preeclampsia, as compared to pregnant control subjects. This rapid and sensitive method seems useful in quantifying podocyturia. The question that remains is: is podocyturia a diagnostic tool to detect preeclampsia during the early stages of pregnancy, i.e. before the onset of clinical symptoms? Recently, a very promising paper was published, in which the authors describe that podocyturia precedes both proteinuria and the clinical features of preeclampsia.[20] In all preeclamptic patients, podocyturia was detected in the second trimester, whereas podocyturia was never observed in normotensive controls, nor in patients who developed gestational hypertension. Podocyturia in the second trimester had a significantly greater sensitivity and specificity (both 100%, providing perfect likelihood ratios) for the subsequent diagnosis of preeclampsia than any single angiogenic factor, such as sFlt-1 or s-Eng, or a combination of these factors. These findings implicate promising possibilities of using podocyturia for accurate identification of pregnant women

at risk for preeclampsia. Importantly, these findings also add to our understanding of the pathophysiologic mechanisms leading to preeclampsia-related proteinuria. Podocyturia was observed in the second trimester before the onset of proteinuria.[20] This may indicate that glomerular loss of podocytes may lead to a disruption of the glomerular filtration barrier and, thereby, to proteinuria. However, it has also been described that proteinuria impairs podocyte regeneration,[45] and it may be speculated that proteinuria therefore leads to podocyturia. Another possibility is that proteinuria and podocyturia are both caused by one upstream mechanism. It could also be the case that proteinuria and podocyturia happen at the same time, but are the result of different underlying mechanisms. Despite the significant loss of podocytes in the urine, the work described in this thesis showed that the number of glomerular podocytes is unaffected in preeclampsia. The higher number of activated glomerular parietal epithelial cells present in kidneys from women with preeclampsia may suggest that lost podocytes are replaced by progenitor cells of the parietal epithelium.

The replacement of lost podocytes by activated parietal epithelial cells could be a favourable phenomenon. In a Munich Wistar Frömter (MWF) rat model of spontaneous glomerular injury, this compensatory mechanism has been described to contribute to remodeling of the glomerular architecture, if successful.[46] Perhaps this mechanism could be responsible for the fact that the glomerular lesions in preeclampsia usually disappear within a few weeks after delivery.[47, 48] However, instead of a repair response by parietal epithelial cells, murine studies have illustrated the important role of parietal epithelial cells in the development of FSGS, by showing that an excessive proliferative response of parietal epithelial cells is involved in the progression of FSGS.[49] In human renal transplants, activated parietal epithelial cells are present in significantly higher numbers in early recurrent FSGS than in minimal change disease.[18] Women with preeclampsia have a higher risk of developing

FSGS later in life[19], but the pathogenesis of this phenomenon is poorly understood. Based on the aforementioned studies, one may hypothesize that an excessive proliferative response of parietal epithelial cells may lead to kidney injury, in particular FSGS.

We found higher numbers of activated parietal epithelial cells in kidneys from women with preeclampsia than in the control groups. Taking the abovementioned studies into consideration, it could be hypothesized that the higher numbers of parietal epithelial cells in the preeclamptic kidney could be a sign of repair, or a sign of progressive podocytopathy. In case the higher numbers of parietal epithelial cells are a sign of progressive podocytopathy, this podocytopathy may be associated with the risk of developing FSGS on the long run. In addition, the replacement of lost podocytes by activated parietal epithelial cells might also be responsible for the increased risk (relative risk of approximately 4) for women with preeclampsia to develop end-stage renal disease.[50] This increased risk could be a direct effect of preeclampsia. However, preeclampsia itself is also associated with an increased risk of cardiovascular disease, and cardiovascular disease is in turn associated with kidney disease.[51] Therefore, it is challenging to distinguish the precise role of preeclampsia in the development of kidney disease. However, the risk of kidney disease and micro-albuminuria one decade after preeclampsia seems to be greater than the risk of cardiovascular disease,[52, 53] which underscores the hypothesis that preeclampsia might induce a primary renal insult.

PATIENTS' PERSPECTIVES

No single factor can be held responsible for the development of kidney disease during and after preeclampsia. A variety of risk factors and mechanisms probably underlies this phenomenon. Regardless of which factors contribute, preeclampsia is an important risk factor for subsequent chronic kidney disease. This notion calls for early detection and intervention. To start with, early prediction of preeclampsia—for example by using podocyturia or urinary metabolomics analysis—could provide an opportunity for close surveillance and preventive strategies, such as early delivery. It lends credence to speculation that targeting factors that contribute to the pathogenesis of kidney disease in preeclampsia may have therapeutic value. For instance, murine models have shown reversal of proteinuria and of histologic changes typical of preeclampsia when treated with complement inhibitors.[54] In humans, Eculizumab—a terminal complement inhibitor—has recently been administered in a woman with preeclampsia. This resulted in abrogation of preeclampsia manifestations and prolongation of pregnancy.[55] Further research is warranted to validate these findings in a larger clinical study, especially regarding the potential side effects of the treatment. In addition, several studies showed that targeting the excessive proliferative response of parietal epithelial cells[56] can be achieved by therapies, such as ACE-inhibitors[57] and inhibitors of the Notch-signaling pathway.[58] Similarly to Eculizumab, ACE-inhibitors, and Notch-signaling pathway inhibitors should be further studied in randomised controlled trials, in particular regarding the side effects for the mother and the fetus. Furthermore, long-term follow-up is recommended after delivery, especially with respect to hypertension, obesity, and insulin resistance.[51] Whether monitoring of these risk factors and usage of the abovementioned therapeutic options will improve the renal condition during and after preeclampsia and might decrease the risk of kidney disease later in life, should be subject of future studies.

References

1. Levine RJ, Maynard SE, Qian C, Lim K-H, England LJ, Yu KF, et al. Circulating angiogenic factors and the risk of preeclampsia. *New Engl J Med*. 2004;350:672-683
2. Rajakumar A, Cerdeira AS, Rana S, Zsengeller Z, Edmunds L, Jeyabalan A, et al. Transcriptionally active syncytial aggregates in the maternal circulation may contribute to circulating soluble fms-like tyrosine kinase 1 in preeclampsia. *Hypertension*. 2012
3. Bianchi DW, Zickwolf GK, Weil GJ, Sylvester S, DeMaria MA. Male fetal progenitor cells persist in maternal blood for as long as 27 years postpartum. *Proc Natl Acad Sci U S A*. 1996;93:705-708
4. Bianchi DW. Robert e gross lecture. Fetomaternal cell trafficking: A story that begins with prenatal diagnosis and may end with stem cell therapy. *J Pediatr Surg*. 2007;42:12-18
5. Kremer Hovinga IC, Koopmans M, de Heer E, Bruijn JA, Bajema IM. Chimerism in systemic lupus erythematosus--three hypotheses. *Rheumatology (Oxford)*. 2007;46:200-208
6. Berger SP, Roos A, Daha MR. Complement and the kidney: What the nephrologist needs to know in 2006? *Nephrol Dial Transplant*. 2005;20:2613-2619
7. Buurma A, Cohen D, Veraar K, Schonkeren D, Claas FH, Bruijn JA, et al. Preeclampsia is characterized by placental complement dysregulation. *Hypertension*. 2012;60:1332-1337
8. Sinha D, Wells M, Faulk WP. Immunological studies of human placentae: Complement components in pre-eclamptic chorionic villi. *Clin Exp Immunol*. 1984;56:175-184
9. Tedesco F, Radillo O, Candussi G, Nazzaro A, Mollnes TE, Pecorari D. Immunohistochemical detection of terminal complement complex and s protein in normal and pre-eclamptic placentae. *Clin Exp Immunol*. 1990;80:236-240
10. Lynch AM, Murphy JR, Byers T, Gibbs RS, Neville MC, Giclas PC, et al. Alternative complement pathway activation fragment bb in early pregnancy as a predictor of preeclampsia. *Am J Obstet Gynecol*. 2008;198:385 e381-389
11. Fu M, Fan PS, Li W, Li CX, Xing Y, An JG, et al. Identification of poly-reactive natural igm antibody that recognizes late apoptotic cells and promotes phagocytosis of the cells. *Apoptosis*. 2007;12:355-362
12. Vollmers HP, Brandlein S. Natural human immunoglobulins in cancer immunotherapy. *Immunotherapy*. 2009;1:241-248
13. van der Pol P, Roos A, Berger SP, Daha MR, van Kooten C. Natural igm antibodies are involved in the activation of complement by hypoxic human tubular cells. *Am J Physiol Renal Physiol*. 2011;300:F932-940
14. Peng Y, Kowalewski R, Kim S, Elkon KB. The role of igm antibodies in the recognition and clearance of apoptotic cells. *Mol Immunol*. 2005;42:781-787
15. Strassheim D, Renner B, Panzer S, Fuquay R, Kulik L, Ljubanovic D, et al. Igm contributes to glomerular injury in fsgs. *J Am Soc Nephrol*. 2013;24:393-406
16. Brewer JW, Randall TD, Parkhouse RM, Corley RB. Mechanism and subcellular localization of secretory igm polymer assembly. *J Biol Chem*. 1994;269:17338-17348
17. Wallukat G, Homuth V, Fischer T, Lindschau C, Horstkamp B, Jupner A, et al. Patients with preeclampsia develop agonistic autoantibodies against the angiotensin at1 receptor. *J Clin Invest*. 1999;103:945-952
18. Fatima H, Moeller MJ, Smeets B, Yang H, D'Agati VD, Alpers CE, et al. Parietal epithelial cell activation marker in early recurrence of fsgs in the transplant. *Clin J Am Soc Nephrol*. 2012;7:1852-1858
19. Vikse BE, Hallan S, Bostad L, Leivestad T, Iversen BM. Previous preeclampsia and risk for progression of biopsy-verified kidney disease to end-stage renal disease. *Nephrol Dial Transplant*. 2010;25:3289-3296
20. Craici IM, Wagner SJ, Bailey KR, Fitz-Gibbon PD, Wood-Wentz CM, Turner ST, et al. Podocyturia predates proteinuria and clinical features of preeclampsia: Longitudinal prospective study. *Hypertension*. 2013;61:1289-1296
21. Holmes E, Wilson ID, Nicholson JK. Metabolic phenotyping in health and disease. *Cell*. 2008;134:714-717
22. Akolekar R, Syngelaki A, Sarquis R, Zvanca M, Nicolaides KH. Prediction of early, intermediate and late pre-eclampsia from maternal factors, biophysical and biochemical markers at 11-13 weeks. *Prenat Diagn*. 2011;31:66-74
23. Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. *Nat Med*. 1995;1:27-31
24. Maharaj AS, D'Amore PA. Roles for vegf in the adult. *Microvasc Res*. 2007;74:100-113
25. Steegers EA, von Dadelszen P, Duvekot JJ, Pijnenborg R. Pre-eclampsia. *Lancet*. 2010;376:631-644
26. Benigni A, Gaspari F, Orisio S, Bellizzi L, Amuso G, Frusca T, et al. Human placenta expresses endothelin gene and corresponding protein is excreted in urine in increasing amounts during normal pregnancy. *Am J Obstet Gynecol*. 1991;164:844-848
27. Gerbasi FR, Bottoms S, Farag A, Mammen E. Increased intravascular coagulation associated with pregnancy. *Obstet Gynecol*. 1990;75:385-389
28. Potter JM, Nestel PJ. The hyperlipidemia of pregnancy in normal and complicated pregnancies. *Am J Obstet Gynecol*. 1979;133:165-170
29. Venkatesha S, Toporsian M, Lam C, Hanai J, Mammoto T, Kim YM, et al. Soluble endoglin contributes to the pathogenesis of preeclampsia. *Nat Med*. 2006;12:642-649
30. Buurma AJ, Turner RJ, Driessen JH, Mooyaart AL, Schoones JW, Bruijn JA, et al. Genetic variants in pre-eclampsia: A meta-analysis. *Hum Reprod Update*. 2013;19:289-303
31. Irgens HU, Reisaeter L, Irgens LM, Lie RT. Long term mortality of mothers and fathers after pre-eclampsia: Population based cohort study. *BMJ*. 2001;323:1213-1217
32. Barden A. Pre-eclampsia: Contribution of maternal constitutional factors and the consequences for cardiovascular health. *Clin Exp Pharmacol Physiol*. 2006;33:826-830
33. Ramsay JE, Stewart F, Greer IA, Sattar N. Microvascular dysfunction: A link between pre-eclampsia and maternal coronary heart disease. *Br J Obstet Gynaecol*. 2003;110:1029-1031
34. Kenny LC, Broadhurst DI, Dunn W, Brown M, North Ra, McCowan L, et al. Robust early pregnancy prediction of later preeclampsia using metabolomic biomarkers. *Hypertension*. 2010;56:741-749
35. Maynard SE, Min J, Merchan J, Lim K, Li J, Mondal S, et al. Excess placental soluble fms-like tyrosine kinase 1 (sflt1) may contribute to endothelial dysfunction , hypertension , and proteinuria in preeclampsia. *J Clin Invest*. 2003;111:649-658
36. Walport MJ. Complement. First of two parts. *N Engl J Med*. 2001;344:1058-1066
37. Walport MJ. Complement. Second of two parts. *N Engl J Med*. 2001;344:1140-1144
38. Girardi G, Yarilin D, Thurman JM, Holers VM, Salmon JE. Complement activation induces dysregulation of angiogenic factors and causes fetal rejection and growth restriction. *J Exp Med*. 2006;203:2165-2175
39. Keir LS, Welsh G, Coward R, Richards A, Spooner RA, Saleem M. The podocyte is the initial target in the renal pathogenesis of diarrhhoea-associated haemolytic uraemic syndrome [abstract]. *SA-PO2344*. ASN Kidney Week 2011
40. Rougier N, Kazatchkine MD, Rougier JP, Fremeaux-Bacchi V, Blouin J, Deschenes

G, et al. Human complement factor h deficiency associated with hemolytic uremic syndrome. *J Am Soc Nephrol*. 1998;9:2318-2326
41. Stahl AL, Vaziri-Sani F, Heinen S, Kristoffersson AC, Gydell KH, Raafat R, et al. Factor h dysfunction in patients with atypical hemolytic uremic syndrome contributes to complement deposition on platelets and their activation. *Blood*. 2008;111:5307-5315
42. Del Conde I, Cruz MA, Zhang H, Lopez JA, Afshar-Kharghan V. Platelet activation leads to activation and propagation of the complement system. *J Exp Med*. 2005;201:871-879
43. Hossain N, Schatz F, Paidas MJ. Heparin and maternal fetal interface: Why should it work to prevent pregnancy complications? *Thromb Res*. 2009;124:653-655
44. Yu D, Petermann A, Kunter U, Rong S, Shankland SJ, Floege J. Urinary podocyte loss is a more specific marker of ongoing glomerular damage than proteinuria. *J Am Soc Nephrol*. 2005;16:1733-1741
45. Peired A, Angelotti ML, Ronconi E, la Marca G, Mazzinghi B, Sisti A, et al. Proteinuria impairs podocyte regeneration by sequestering retinoic acid. *J Am Soc Nephrol*. 2013;24:1756-1768
46. Macconi D, Sangalli F, Bonomelli M, Conti S, Condorelli L, Gagliardini E, et al. Podocyte repopulation contributes to regression of glomerular injury induced by ace inhibition. *Am J Pathol*. 2009;174:797-807
47. Heaton JM, Turner DR. Persistent renal damage following pre-eclampsia: A renal biopsy study of 13 patients. *J Pathol*. 1985;147:121-126
48. Kincaid-Smith P. The renal lesion of preeclampsia revisited. *Am J Kidney Dis*. 1991;17:144-148
49. Smeets B, Kuppe C, Sicking EM, Fuss A, Jirak P, van Kuppevelt TH, et al. Parietal epithelial cells participate in the formation of sclerotic lesions in focal segmental glomerulosclerosis. *J Am Soc Nephrol*. 2011;22:1262-1274
50. Vikse BE, Irgens LM, Leivestad T, Skjaerven R, Iversen BM. Preeclampsia and the risk of end-stage renal disease. *New Engl J Med*. 2008;359:800-809
51. Vikse BE. Pre-eclampsia and the risk of kidney disease. *Lancet*. 2013;382:104-106
52. Bellamy L, Casas JP, Hingorani AD, Williams DJ. Pre-eclampsia and risk of cardiovascular disease and cancer in later life: Systematic review and meta-analysis. *BMJ*. 2007;335:974
53. McDonald SD, Han Z, Walsh MW, Gerstein HC, Devereaux PJ. Kidney disease after preeclampsia: A systematic review and meta-analysis. *Am J Kidney Dis*. 2010;55:1026-1039
54. Qing X, Redecha PB, Burmeister Ma, Tomlinson S, D'Agati VD, Davisson RL, et al. Targeted inhibition of complement activation prevents features of preeclampsia in mice. *Kidney Int* 2011;79:331-339
55. Burwick RM, Feinberg BB. Eculizumab for the treatment of preeclampsia/hellp syndrome. *Placenta*. 2013;34:201-203
56. Moeller MJ, Smeets B. Novel target in the treatment of rpgn: The activated parietal cell. *Nephrol Dial Transplant*. 2013;28:489-492
57. Benigni A, Morigi M, Rizzo P, Gagliardini E, Rota C, Abbate M, et al. Inhibiting angiotensin-converting enzyme promotes renal repair by limiting progenitor cell proliferation and restoring the glomerular architecture. *Am J Pathol*. 2011;179:628-638
58. Lasagni L, Ballerini L, Angelotti ML, Parente E, Sagrinati C, Mazzinghi B, et al. Notch activation differentially regulates renal progenitors proliferation and differentiation toward the podocyte lineage in glomerular disorders. *Stem Cells*. 2010;28:1674-1685