IV Association of preeclampsia with podocyte turnover

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Clinical Journal of the American Society of Nephrology,
in press

Abstract

BACKGROUND AND OBJECTIVES

Preeclampsia is a pregnancy-related complication that causes significant perinatal and maternal morbidity and mortality worldwide. Preeclampsia is characterized by hypertension and proteinuria, and increased shedding of podocytes into the urine is a common finding. This finding raises the question of whether preeclamptic nephropathy involves podocyte damage. We therefore studied podocyte-related changes in a unique sample of renal tissues obtained from women who died of preeclampsia.

DESIGN, SETTING, PARTICIPANTS AND MEASUREMENTS

Using a nationwide database of the Dutch Pathology Registry (PALGA), we identified a cohort of 11 women who died from preeclampsia. Three control groups were also identified and consisted of normotensive women who died during pregnancy (n=25), and non-pregnant controls either with (n=14) or without (n=13) chronic hypertension. Clinical data regarding the pregnant patients were obtained from the National Maternal Mortality Committee of the Dutch Society of Obstetrics and Gynaecology. Renal tissues were obtained. Glomerular lesions, including podocyte numbers, podocyte proliferation, and parietal cell activation were measured and compared between the four groups.

RESULTS

The preeclamptic patients had prominent characteristic glomerular lesions, including endothelial cell swelling, podocyte swelling, and double contours of the glomerular basement membrane. Importantly, we found that the number of podocytes per glomerulus did not differ significantly between the preeclamptic and control groups. However, preeclampsia was associated with a significant increase in intraglomerular cell proliferation (p=0.004) and activated parietal epithelial cells on a podocyte location (p=0.01).

CONCLUSIONS

Our findings suggest that the recently described mechanisms of podocyte replacement play a role in preeclampsia. Our results also suggest that the podocyte plays an important role in preeclamptic nephropathy and subsequent progressive renal damage, including focal and segmental glomerulosclerosis. These findings provide key new insights into the pathogenesis of preeclamptic nephropathy, and they open new possibilities for developing therapeutic modalities.

Introduction

Preeclampsia is a serious pregnancy-related complication that affects up to 8% of all pregnancies, thereby causing significant perinatal and maternal morbidity and mortality worldwide.[1] Preeclampsia is believed to arise–at least in part–from an imbalance between the pro- and antiangiogenic factors in the maternal circulation; the maternal kidney is particularly sensitive to this imbalance, as reflected by the occasional finding of severe proteinuria.[1, 2]

As far back as 1918, the glomerulus was recognized as the principal site of renal damage in preeclampsia.[3] Later, the glomerular podocyte became the focus of attention in relation to proteinuria. Because renal biopsies are rarely performed in pregnant preeclamptic patients, how the podocyte is affected by preeclampsia has remained largely unknown, although endotheliosis is generally considered a characteristic histopathological glomerular lesion in preeclampsia. Recently, Vikse[4] suggested that a previously undetected renal disease might become “overt” in the preeclamptic setting. Preeclampsia is a major risk factor for developing chronic kidney disease - in particular, focal and segmental glomerulosclerosis (FSGS), which is considered primarily a disease of podocytes - later in life. However, how preeclampsia increases the risk of renal disease is poorly understood.

Recently, our group and others reported significantly higher

numbers of podocytes in the urine (i.e. podocyturia) of women with preeclampsia compared to pregnant control subjects.[5, 6] This podocyturia remained in the preeclamptic patients up to one month after delivery, although their proteinuria resolved.[7] Structural changes in the podocyte, including abnormal expression of podocyte-related proteins, were recently reported in a limited number of patients with preeclampsia.[8, 9] These findings suggest that the podocyte plays a key role in preeclampsia. In particular, the increased shedding of podocytes into the urine of preeclamptic patients raises the question of whether the origin of preeclamptic renal disease involves podocyte damage. Moreover, this hypothesis is reminiscent of the podocytopathy that may underlie the subsequent renal deterioration that is seen in FSGS. To investigate the association between preeclampsia and glomerular lesions in general, and podocyte-related injury in particular, we collected a unique sample of renal autopsy tissues obtained from preeclamptic patients and control subjects.

Methods

PATIENT SELECTION AND NATIONWIDE PALGA SEARCH FOR RENAL TISSUE

Autopsy samples were obtained following a nationwide search of the Dutch Pathology Registry (PALGA), a histopathology and cytopathology network and registry that includes all pathology laboratories within the Netherlands.(10) We included pregnant patients who died from preeclampsia as defined by international guidelines established by the International Society for the Study of Hypertension in Pregnancy (ISSHP).[11] Three control groups were also obtained. One control group consisted of pregnant women without a hypertensive disorder either prior to, or during the pregnancy. The two other control groups consisted of non-pregnant young women either with, or without a medical history

of chronic hypertension. We obtained paraffin-embedded kidney samples taken from 11 preeclampsia patients, 25 normotensive pregnant controls, 14 chronic hypertensive non-pregnant controls, and 13 normotensive non-pregnant controls. The patients' clinical characteristics were obtained from their autopsy-reports. The cause of death in each pregnant case was confirmed by reviewing the records of the National Maternal Mortality Committee of the Dutch Society of Obstetrics and Gynecology.[12] A thorough review of the autopsy reports— including kidney weight—confirmed that the control subjects had no evidence of underlying renal disease. All tissues were coded and handled anonymously in accordance with the Dutch National Ethics Guidelines (Code for Proper Secondary Use of Human Tissue, Dutch Federation of Medical Scientific Societies). This study was approved by the ethics committee of the Leiden University Medical Center (P12.107).

HISTOLOGY, IMMUNOHISTOCHEMISTRY, AND IMMUNOFLUORESCENCE

Sections of renal tissue samples were stained with haematoxylin and eosin, periodic acid-Schiff (PAS), silver, and phosphotungstic acid-hematoxylin (PTAH) using standard methods. Immunohistochemistry was used to identify and count podocytes based on staining for WT-1, a podocyte-specific transcription factor.[13] To confirm the origin of swollen endothelial cells, we performed CD31 staining experiments. Because cell proliferation can affect the number of podocytes, we also performed immunohistochemical staining for Ki-67, a marker of cell proliferation.[14] The sections were deparaffinized, and after antigen retrieval, immunohistochemistry was performed. The sections were stained with antibodies against WT-1 (rabbit anti-human polyclonal antibody sc-192 lotnumber D2104, Santa Cruz Biotechnology, 1:250), or CD31 (Dako, 1:400) or Ki-67 (Thermo Fisher Scientific, 1:200), and staining was visualized using the appropriate secondary antibodies and diaminobenzidine

as the chromagen. Finally, the sections were counterstained with hematoxylin.

Double immunofluorescence stainings were performed. For the first double staining, we used an antibody against CD44 (a glycoprotein involved in cell adhesion, cell matrix interactions, and cell migration (Abcam, 1:200)), which is expressed by activated parietal epithelial cells[12] and an antibody against CD45 (a leukocyte common antigen, Ancell 1:800). Staining was visualized using goat anti-mouse Alexa 488 IgG2a and Alexa 546 Ig1. For a second double-staining experiment, we used antibodies against CD44 (Abcam, 1:200) and Ki-67 (Thermo Fisher Scientific, 1:200). Staining was visualized using the goat anti-mouse Alexa 488 IgG2 (1:200) and Alexa 546 Ig1 (1:200) secondary antibodies. We also performed a third double-staining experiment using antibodies against WT-1 (Santa Cruz Biotechnology, 1:250) and CD44 (Abcam, 1:200). WT-1 was visualized using the appropriate secondary antibody and diaminobenzidine as the chromagen, and CD44 was visualized using the FITC-labelled goat anti-mouse IgG antibody (1:200).

QUANTIFICATION OF HISTOLOGY

Sections were examined and scored by an experienced renal pathologist who was blinded with respect to the patients' clinical data. At least 50 glomeruli per section were examined, and the following parameters were scored: presence of endotheliosis, double contours of the glomerular basement membrane (tram tracking), swelling of podocytes, mesangial changes, glomerulitis, and focal and segmental glomerulosclerosis (FSGS). As described by previously Strevens et al[15], endotheliosis was scored semi-quantitatively as follows: 0 (no endotheliosis), 1 (<20% of the lumen was obliterated), 2 (20-80% of the lumen was obliterated), or 3 (>80% of the lumen was obliterated). Global sclerosis was recorded as a percentage of the total number of glomeruli scored. PTAH-confirmed microthrombi, interstitial fibrosis and tubular atrophy (IFTA), and acute tubular necrosis (ATN) were scored as either absent or present. To quantify vessel changes, the presence of hyalinosis and intimal fibrosis of arteries was scored. Finally, signs of ischemia, congestion, and edema were also evaluated.

MORPHOMETRY AND QUANTIFICATION OF IMMUNOHISTOCHEMISTRY AND IMMUNOFLUORESCENCE

Because differences between the groups with respect to glomerular surface areas can affect the absolute number of podocytes, PAS-stained slides were used to measure the surface areas of the glomerular tuft and Bowman's capsule of 30 randomly selected glomeruli (using ImageJ 1.47d software; National Institutes of Health, downloaded from http://rsb.info.nih.gov/ij). For the immunohistochemical and immunofluorescence stained sections, at least 30 glomeruli per section were analyzed by two observers who were blinded with respect to the patients' clinical data. The number of WT-1 positive nuclei per glomerular cross-section was counted using ImageJ 1.47d. Randomly selected regions of the outer renal cortex were analyzed. Because estimates of podocyte number vary widely between studies[16], we measured podocytes in a control group containing non-pregnant, non-hypertensive women as a measure of the number of podocytes per glomerular cross section in healthy adult women. Ki-67 staining was quantified by counting the number of Ki-67-positive cells within the glomeruli (both intraglomerular and lining Bowman's capsule). Finally, the presence of CD44-positive cells in the glomeruli was scored along the inner lining of Bowman's capsule—in an anatomic parietal epithelial cell location—and covering the glomerular basement membrane on a podocyte location (i.e., not counting endothelial cells) as previously described by Fatima *et al*[17]. The podocyte location was confirmed by co-localization between CD44 and WT-1. In addition, the number of cellular bridges (i.e., bridges between Bowman's capsule and the glomerular tuft) was scored in both CD44-stained and silver-stained samples. CD44-positive leukocytes, which were confirmed by co-staining with CD45, were excluded from the scoring analysis.

STATISTICAL ANALYSIS

Categorical variables were compared using the Chi-square test. Differences in quantitative parameters between groups were assessed using either the one-way ANOVA (for normally distributed data) or the non-parametric Kruskal-Wallis test (for non-normally distributed data). Correlations were calculated using either a Spearman's (for ordinal data) or Pearson's (for numerical data) coefficient. All analyses were performed using the SPSS statistical software package (version 20.0; Armonk, NY: IBM Corp). Differences with a *p*-value less than 0.05 were considered statistically significant.

Results

CLINICAL DATA

The clinical characteristics of the four groups are summarized in Table 1. The hypertensive non-pregnant control group was significantly (p=0.03) older than the other study groups. No other significant differences were observed with respect to the other clinical characteristics.

CHARACTERISTIC RENAL HISTOLOGY FINDINGS IN PREECLAMPSIA

The majority (81%) of the women with preeclampsia had prominent glomerular lesions, including various degrees of endotheliosis, swelling of podocytes, and tram tracking. Endotheliosis was present in 55% of the women with preeclampsia. Although endotheliosis is generally considered to be the principal feature of preeclamptic glomerular changes, it was also observed - albeit to a significantly lesser extent (p=0.003) - in both the pregnant control group (12%) and the hypertensive non-pregnant control group (15%). Both tram tracking (p<0.001) and podocyte swelling (p=0.02) were present in the preeclampsia group only. With the exception of one patient with preeclampsia, endotheliosis and tram tracking were not present simultaneously. The presence of podocyte changes was correlated significantly with the presence of endotheliosis (p=0.001). The presence of endotheliosis was not correlated with blood pressure, proteinuria, gestational age, or maternal age. No correlation was found between renal histopathological lesions and gestational age. The hypertensive non-pregnant control subjects had significantly more severe ischemic glomerular lesions than the preeclamptic patients; these and other lesions are summarized in Table 2. Supplemental Figures S1 and S2 show typical examples of renal histology from all four study groups.

MORPHOMETRIC ANALYSES

Glomerular surface areas did not differ significantly between the preeclampsia and control groups. However, the chronic hypertensive non-pregnant control subjects had significantly larger surface areas in the glomerular tuft and Bowman's capsule than the normotensive non-pregnant control subjects (Figure 1A and B). Similar results were obtained when we calculated glomerular volume using the Weibel-Gomez method.[18]

THE PREECLAMPSIA PATIENTS AND CONTROL GROUPS HAVE SIMILAR NUMBERS OF PODOCYTES

No significant difference in the number of podocytes was found between the preeclamptic group and the control groups (Figure 1C). Supplemental Figure S3A shows a typical example of WT-1 staining. WT-1 staining was not correlated with either the renal histopathological lesions or patient characteristics.

PREECLAMPSIA IS ASSOCIATED WITH INCREASED CELLULAR PROLIFERATION

The women with preeclampsia had significantly more intraglomerular Ki-67-positive cells compared to the hypertensive and normotensive non-pregnant control groups (p=0.004; Figure 2A). Furthermore, the women with preeclampsia had significantly more Ki-67-positive parietal epithelial cells than the pregnant controls and the hypertensive non-pregnant control subjects (p=0.02; Figure 2B). Supplemental Figure S3B shows a typical example of Ki-67 staining. Ki-67 and CD44 staining was co-localized (an example is shown in Supplemental Figure S4). Ki-67 staining was not correlated with either the renal histopathological lesions or patient characteristics.

PREECLAMPSIA IS ASSOCIATED WITH AN INCREASED NUMBER OF CD44 POSITIVE PARIETAL CELLS ON A PODOCYTE LOCATION

We scored the presence and location of CD44-positive/ CD45-negative cells within the glomeruli for the four study groups (Figure 3A-C). The podocyte location was confirmed by co-localization of CD44 and WT-1 staining (an example is shown in Figure S5A and B). The number of CD44-positive cells on a podocyte location was significantly higher in the women with preeclampsia than in all three control groups (Figure 3D). Moreover, the presence of CD44-positive cells was significantly associated with the presence of Ki-67-positive intraglomerular cells (p=0.03). Although a trend was observed between preeclampsia and CD44-positive cells on a parietal epithelial cell location, this association did not reach statistical significance (p=0.07; Figure 3E). The number of cellular bridges did not differ significantly between the study groups; however, the presence of cellular bridges was significantly associated with the presence of FSGS (p=0.02). An example of a cellular bridge is shown in Supplemental Figure S6.

Discussion

In our cohort of preeclamptic women, podocyte changes and tram tracking of the glomerular basement membrane were the most typical preeclampsia-associated lesions, occurring in 18% and 36% of patients, respectively; in contrast, none of the patients in our control groups had either of these lesions. Endotheliosis, a lesion that has been previously described as a characteristic of preeclampsia, was present in 55% of the preeclampsia patients, but it was also present – albeit at much lower percentages - in the pregnant controls and the hypertensive non-pregnant controls. We also provide the first report that although the number of glomerular podocytes is unaffected in preeclampsia (as determined by histological evaluation), preeclampsia is characterized by a higher number of activated parietal epithelial cells. A mechanism to explain our findings might come from elegant experiments by Appel et al., who used a rat model for lineage tracing and gene tagging and found that parietal epithelial cells migrate into the glomerular tuft.(19) Moreover, our findings strongly suggest that lost podocytes are replaced by progenitor cells of the parietal epithelium in the context of preeclampsia. Importantly, none of the patients had any indication of underlying renal disease, suggesting that these findings are likely attributable to preeclampsia.

Preeclampsia is characterized by an increase in the shedding of podocytes into the urine,[5-7] and this can occur before the onset of clinical manifestations.[20] Furthermore, the expression of podocyte-specific proteins such as nephrin and GLEPP-1 is significantly lower in kidney biopsies from patients with preeclampsia than from control subjects.[8, 9] Given the increased shedding of podocytes, our current findings that the number of glomerular podocytes remains stable suggests an increased turnover of podocytes in preeclampsia. Podocytes have traditionally been regarded as highly differentiated, non-dividing cells, which would imply that these cells cannot regenerate following podocyte injury and/ or loss. However, a

recent study reported that the parietal epithelial cells that line Bowman's capsule can replace injured and lost podocytes.[19] On the other hand, a recent mouse study reported that proteinuria inhibits the differentiation of parietal epithelial cells into podocytes by sequestering retinoic acid.[21]

Recently, it has been shown that parietal epithelial cells – but not podocytes – upregulate their de novo expression of CD44 (marker of cell migration) following podocyte injury and/ or loss.[22, 23] CD44 can also be expressed by endothelial cells.[24] However, in our study CD44 co-localized with WT-1, and CD44 positivity on a podocyte location was significantly higher in the preeclampsia patients than in the three control groups. Based on the aforementioned study describing parietal epithelial cell migration in a rat model [19], our findings suggest that during preeclampsia, activated parietal epithelial cells can migrate and replace lost podocytes. Additional data to support this mechanism included the increased cell proliferation and the co-localization of Ki-67 and CD44 staining that was observed in the glomeruli of the women with preeclampsia. Together with the observed co-localization between WT-1 and CD44, these findings confirm that the Ki-67-positive cells present on a podocyte location are indeed podocytes.

The replacement of lost podocytes by activated parietal epithelial cells is a compensatory mechanism that - if successful - is accompanied by remodeling the glomerular architecture.[25] However, under certain conditions, this replacement mechanism cannot compensate fully, thereby leading to renal damage that is histologically characterized by focal and segmental glomerulosclerosis. For example, in a mouse model of focal and segmental glomerulosclerosis,[23] an excessive proliferative response of parietal epithelial cells was involved in the progression of sclerotic lesions.[22, 23, 26] There is also evidence that in renal transplants, increased CD44 staining – an indicator of activated parietal epithelial cell - distinguishes early recurrent FSGS (which manifests with podocyte foot process effacement only) from

minimal change disease.[17] These authors speculated that CD44 expression in nephrotic patients without sclerosis has positive value in predicting progressive podocyte damage, including FSGS.[17] In our study, the patients with preeclampsia did not have significantly more FSGS in their kidneys; nevertheless, the significant correlation between FSGS and CD44-positive cellular bridges (connecting Bowman's capsule and the glomerular tuft) supports the previous notion that CD44 positive cells are involved in the formation of sclerotic lesions.[17, 23] The results reported by Fatima et al.[17] provide insight into the implications of our current findings, as women with preeclampsia have a higher risk of developing FSGS later in life[27]; moreover, we found that the preeclamptic women in our study had higher CD44 positivity on a podocyte location compared to the control groups. We therefore speculate that this increased CD44 positivity in preeclamptic kidneys is a sign of progressive podocytopathy and may have predictive value for FSGS in the long run. Our findings within the preeclamptic kidney during pregnancy might also explain the reported increased risk for women with preeclampsia to develop end-stage renal disease later in life.(4, 28) However, it bears mentioning that because we investigated autopsy material, we were unable to analyze any possible correlation between CD44 positivity during pregnancy and the development of sclerotic lesions later in life. The sequence of events and the aforementioned putative mechanisms that underlie podocyte replacement and subsequent podocytopathy during preeclampsia are illustrated in Figure 4.

An important lingering question is what early mechanism causes podocyte injury and loss during preeclampsia. In our study, preeclampsia was characterized by damage to all three layers of the glomerular filtration barrier. Consistent with a previous report, although endotheliosis was observed – albeit relatively rarely - in the pregnant controls and hypertensive non-pregnant controls, endotheliosis was most prevalent among the women with preeclampsia.[15] A dysregulation of—and the resulting imbalance

between—proangiogenic and antiangiogenic factors is believed to cause endotheliosis; in particular, increased levels of the antiangiogenic factor sFlt-1 (soluble Fms-like tyrosine kinase) can prevent vascular endothelial growth factor (VEGF) from maintaining the renal endothelium.[29] Because VEGF is essential for the interaction between endothelial cells and podocytes,[30] dysregulation of VEGF also affects the podocyte. The notion that dysregulation of these factors plays a role in the renal manifestations of preeclampsia is supported by studies showing that endotheliosis is a key feature of glomerular injury in a podocyte-specific VEGF-knockout mouse.[29] The same study also reported that similar lesions were observed in patients who were treated with the VEGF inhibitor bevacizumab.[29] Based on these observations, it is highly likely that angiogenic imbalance plays a causative role in the renal manifestations of preeclampsia. However, preeclampsia may arise from a variety of causative factors other than angiogenic imbalance alone.[31]

In conclusion, building on our previous report that preeclampsia is characterized by increased shedding of podocytes into the urine,[6] we now present the first report that the absolute number of glomerular podocytes is actually unchanged during preeclampsia. This finding might be explained by increased cell proliferation and/or significantly increased numbers of activated parietal epithelial cells, suggesting that these activated cells can migrate to a podocyte location. Our results indicate that podocytopathy plays a central role in preeclamptic nephropathy, thereby contributing – at least in part - to the increased risk of developing FSGS later in life. This notion is consistent with previous reports that podocytopathy plays a role in other forms of FSGS, and it lends credence to speculation that targeting the podocyte may have therapeutic value.[32, 33] Indeed, several studies showed that modulatig podocyte turnover by regulating activation of parietal epithelial cells[34] can be achieved by certain therapies such as ACE inhibitors,[35] and inhibitors of the Notch signalling pathway.[36] Whether the activation of parietal epithelial cells is a mechanism to compensate for ongoing podocyte injury and loss, or whether these cells contribute to glomerular injury - or both - remains to be investigated. Nevertheless, unraveling the mechanisms of podocyte damage and parietal epithelial cell recruitment in the setting of preeclampsia may lead to novel approaches for treating renal injury.

Acknowledgments

The authors would like to thank Floor Luken for her excellent technical support.

Characteristics	PE (n=11)	PC (n=25)	HC (n=14)	NPC (n=13)
Age (years)	32.5 (29.5-35.8)	31.0 (28.3-36.6)	41.8 (34.4-43.5) *	33.5 (24.6-40.0)
Gestational age (weeks)	35.7 (34.1-39.0)	33.4 (16.7-40.0)	NA	NA
Parity (mean (SD))	0.6 (1.0)	0.8 (1.0)	NA	NA
Proteinuria (g/ 24 hours)	0.36 (0.3-6.1)	NA	NA	NA
Blood pressure (mmHg)				
Systolic	160.0 (141.3-191.3) **	125.0 (113.5-137.5)	NA	NA
Diastolic	106.0 (87.5-120.0) ***	90.0 (70.0-90.0)	NA	NA
Antihypertensive therapy (n (%))	4 (36)	NA	4 (100) #	NA
Comorbidities (n (%))				
Sickelcell anemia	0 (0)	1 (4)	0 (0)	0 (0)
WPW syndrome	0 (0)	1 (4)	0 (0)	0 (0)
Asthma	0 (0)	2 (8)	0 (0)	0 (0)
Hyperhomocysteinemia	0 (0)	2 (8)	0 (0)	0 (0)
Hypertension	1 (9)	0 (0)	0 (0)	0 (0)
Epilepsia	1 (9)	0 (0)	0 (0)	0 (0)
Mammacarcinoma	0 (0)	0 (0)	1 (7)	0 (0)
Renal insufficiency	0 (0)	0 (0)	2 (14)	0 (0)
Depression	0 (0)	0 (0)	2 (14)	0 (0)
Obesity	0 (0)	0 (0)	3 (21)	1 (8)
Cause of death (n (%))				
Preeclampsia related	11 (100)	0 (0)	0 (0)	0 (0)
EUG	0 (0)	1 (4)	0 (0)	0 (0)
Thromboembolism	0 (0)	6 (24)	2 (14)	0 (0)
Amniotic fluid embolism	0 (0)	2 (8)	0 (0)	0 (0)
Arrhythmias	0 (0)	1 (4)	0 (0)	0 (0)
Cardiac arrest	0 (0)	1 (4)	2 (14)	0 (0)
Eci	0 (0)	2 (8)	1 (7)	0 (0)
Malignancy	0 (0)	1 (4)	1 (7)	0 (0)
Aortadissection	0 (0)	3 (12)	4 (29)	0 (0)
Infection	0 (0)	5 (20)	1 (7)	0 (0)
Cardiomyopathy	0 (0)	2 (8)	0 (0)	0 (0)
Pheochromocytoma	0 (0)	1 (4)	0 (0)	0 (0)
Cerebral bleeding	0 (0)	0 (0)	3 (21)	0 (0)
Suicide	0 (0)	0 (0)	0 (0)	5 (38)
High energy trauma	0 (0)	0 (0)	0 (0)	7 (54)
Drowning	0 (0)	0 (0)	0 (0)	1 (8)
Death-autopsy interval (hrs)	18.0 (6.0-32.3)	24.0 (20.5-24.0)	24.0 (12.0-48.0)	24.0 (24.0-60.0)

Table 1 (previous page): Patient characteristics

*Data are given as the median (IQR) unless otherwise specified. PE: preeclampsia, PC: normotensive pregnant controls, HC: hypertensive non-pregnant controls, NPC: normotensive non-pregnant controls. NA: Not applicable. SD: standard deviation. * p =0.03, ** p=0.001, *** p=0.006. # data on hypertensive therapy of the hypertensive controls was available from 4 patients.*

Histological parameters	PE (*n*=11)	PC (*n*=25)	HC (*n*=14)	NPC (*n*=13)	*p*-value
ATN (%)	0 (0)	4 (16)	3 (21)	3 (23)	0.41
Congestion (%)	0 (0)	0 (0)	0 (0)	3 (23)	0.007
Endotheliosis (%)	6 (55)	3 (12)	2 (14)	0 (0)	0.003
<20% of the lumen (%)	1 (17)	3 (100)	1 (50)	NA	
20-80% of the lumen (%)	3 (50)	0	1 (50)	NA	
>80% of the lumen (%)	2 (33)	0	0	NA	
FSGS (%)	1 (9)	2 (8)	5 (36)	0 (0)	0.03
Global sclerosis >1% (%)	1 (9)	0 (0)	5 (36)	1 (8)	0.04
Glomerulitis (%)	0 (0)	6 (24)	1 (7)	2 (15)	0.23
Hyalinosis (%)	1 (9)	4 (16)	7 (50)	10 (77)	<0.001
IFTA (%)	0 (0)	0 (0)	2 (14)	0 (0)	0.06
Intima fibrosis (%)	2 (18)	7 (28)	11 (79)	5 (39)	0.006
Ischemia (%)	0 (0)	1 (4)	3 (21)	1 (8)	0.17
Mesangium changes (%)	1 (9)	0 (0)	4 (29)	1 (8)	0.04
Microthrombi (%)	1 (9)	0 (0)	1 (7)	0 (0)	0.20
Edema (%)	1 (9)	0 (0)	0 (0)	1 (8)	0.34
Podocyte changes (%)	2 (18)	0 (0)	0 (0)	0 (0)	0.02
Tram tracking (%)	4 (36)	0 (0)	0 (0)	0 (0)	<0.001

Table 2: Renal histological parameters in the patients and control groups

Values are expressed as number of patients (%). ATN: acute tubular necrosis, FSGS: focal and segmental glomerulosclerosis. All FSGS cases were classified as the "Not otherwise specified" (NOS) variant. IFTA: interstitial fibrosis tubular atrophy. PE: preeclampsia, PC: normotensive pregnant controls, HC: hypertensive non-pregnant controls, NPC: normotensive non-pregnant controls.

Figure 1

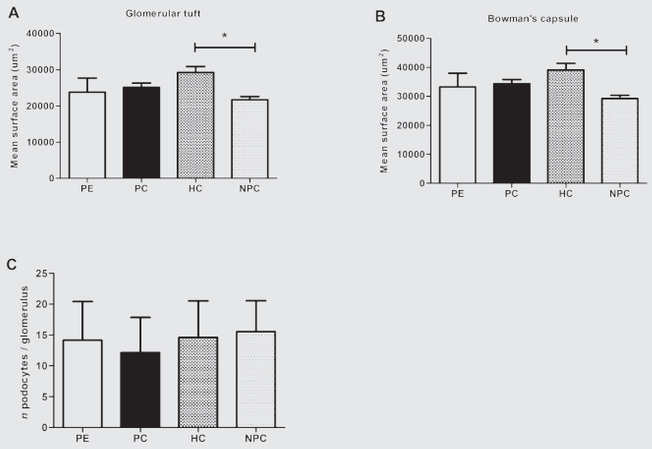


Figure 1: Morphometric analysis and podocyte number

*Glomerular surface areas were calculated for the glomerular tuft (A, * p=0.003) and Bowman's capsule (B, * p=0.002) in the preeclampsia patients and control groups. Panel C summarizes the number of WT-1 positive podocytes per glomerulus in the women with preeclampsia, and the control groups.*

Figure 2

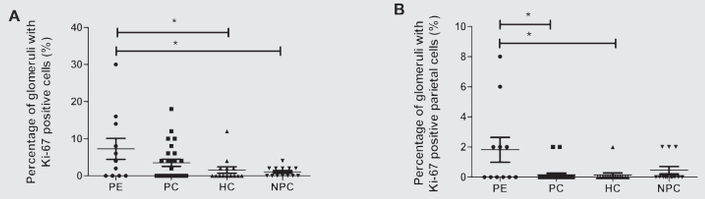
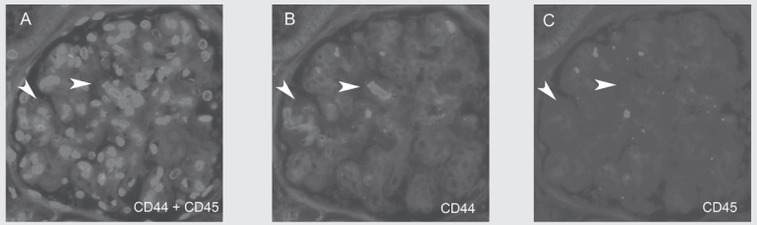


Figure 2: Ki-67 analysis

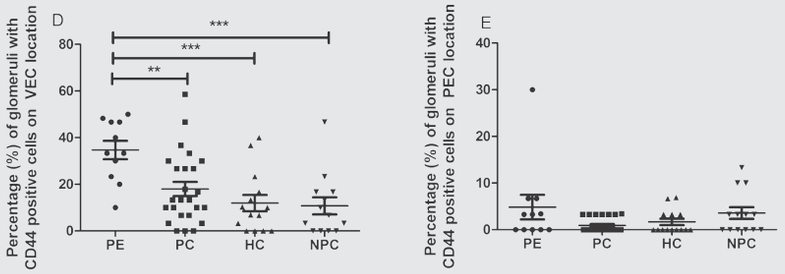
Panel A summarizes the percentage of glomeruli with Ki-67-positive cells (p=0.004), and panel B summarizes the percentage of glomeruli with Ki-67-positive parietal epithelial cells (* p=0.02). In A and B, each symbol represents an individual patient or control.*

Figure 3



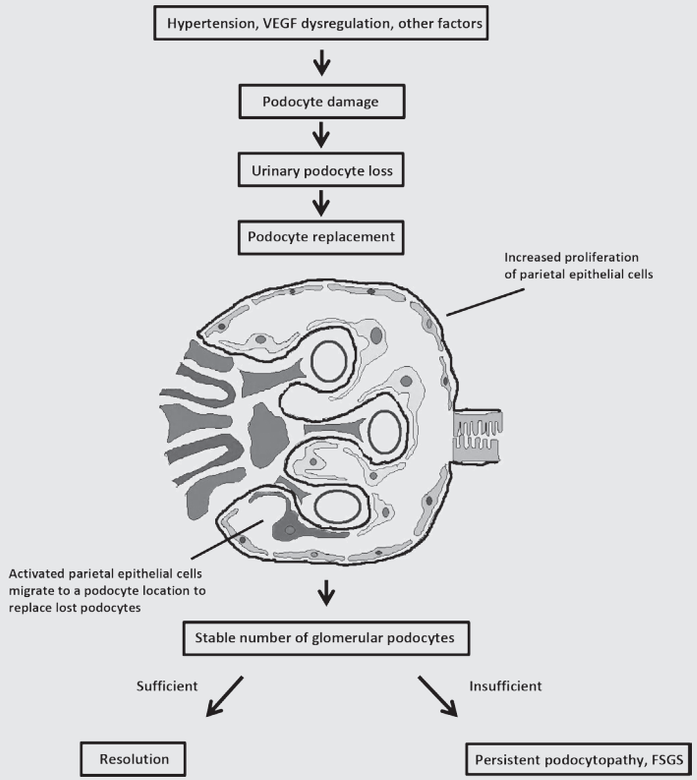
Double staining of CD44 and CD 45 (full colour version inside cover)

Sections were co-stained for CD44 (green) and CD45 (red), and the number of CD44-positive/ CD45-negative cells was scored within the glomeruli. Panel A shows double staining of CD44 positive/ CD45 negative cells on a podocyte location (arrowheads). The nuclei were counterstained with DAPI (blue). Note that the CD44-positive cells (B) are CD45-negative (C).



*Panel D summarizes the number of CD44-positive cells on a podocyte (visceral epithelial cell, VEC) location (** p =0.01, *** p =0.001). Panel E summarizes the number of CD44-positive cells on a parietal epithelial cell location (p=0.07). In D and E, each symbol represents an individual patient or control subject.*

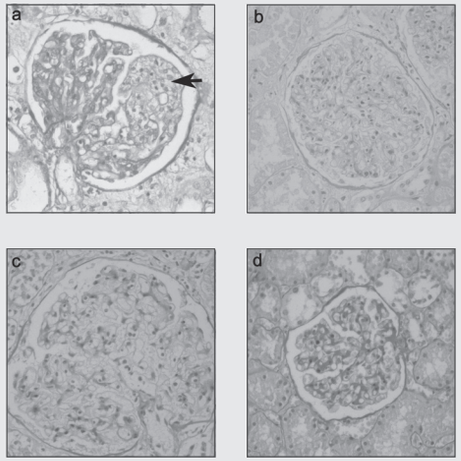
Figure 4



Schematic overview of the putative mechanism underlying podocyte replacement in preeclampsia

Hypertension, dysregulation of VEGF, and other factors cause damage and loss of podocytes during preeclampsia, resulting in increased shedding of podocytes into the urine. In preeclampsia, an increase in turnover of podocytes results from increased proliferation of parietal epithelial cells and higher numbers of activated parietal epithelial cells on a podocyte location. As a result of this increased turnover, the absolute number of glomerular podocytes is stable during preeclampsia. The replacement of lost podocytes by activated parietal epithelial cells is a compensatory mechanism, that – if sufficient – leads to the resolution of clinical symptoms, including proteinuria. However, if compensation is not sufficient, this mechanism can trigger persistent podocytopathy, with progressive proteinuria and renal function loss later in life, histologically characterized by focal and segmental glomerulosclerosis (FSGS).

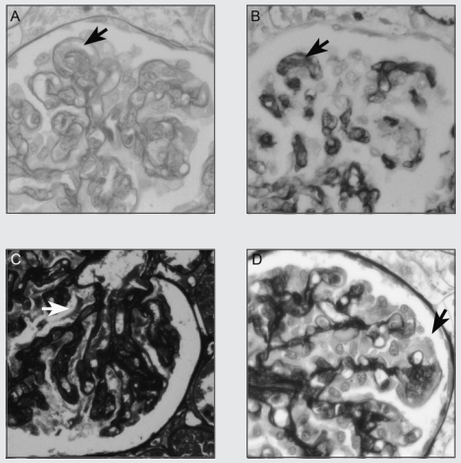
Figure S1



Typical examples of renal histology in preeclampsia and control subjects (full colour version inside cover)

PAS stain showing examples of the various glomerular lesions seen in patients and controls. In preeclamptic patients significantly more endotheliosis (arrow) was observed (a), while the majority of pregnant controls showed no glomerular pathology (b). In hypertensive controls a greater surface area of the glomerular tuft and Bowman's capsule was prominent (c), but non-pregnant controls showed generally normal glomeruli (d).

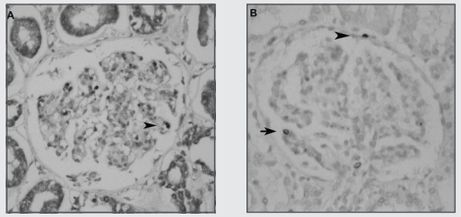
Figure S2



Renal histology in preeclampsia (full colour version inside cover)

This figure shows typical examples of histologic lesions in patients with preeclampsia. In preeclamptic patients significantly more endotheliosis (a, arrow). Note that endotheliosis (a, arrow) consists of endothelial cells, as shown by the positivity for the endothelial marker CD31 (b, arrow). In patient with preeclampsia significantly more tram tracking (c, arrow) was observed than in controls, and podocytes (arrow) were prominently present (d).

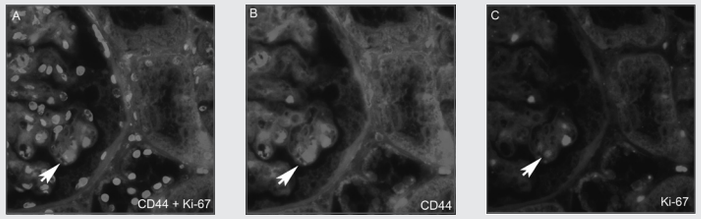
Figure S3



WT-1 and Ki-67 staining (full colour version inside cover)

Panel A shows a typical section with intraglomerular WT-1 positive cells (arrowhead) indicating podocytes. Panel B shows a typical section with Ki-67 positivity, a marker of cell proliferation, in the glomerular tuft (arrow), as well as in parietal epithelial cells (arrowhead).

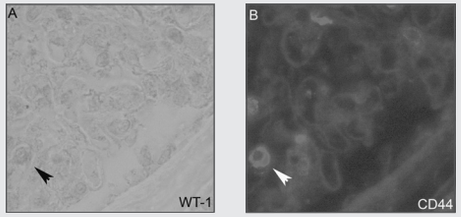
Figure S4



CD44 and Ki-67 staining (full colour version inside cover)

Sections were co-stained for CD44 (green) and Ki-67 (red). Panel A shows double staining of a CD44 positive/ Ki-67 positive cell on a podocyte location (arrow). The nuclei were counterstained with DAPI (blue). Note that the CD44-positive cells shown a membrane staining pattern (B) with Ki-67-positive nuclear staining pattern (C).

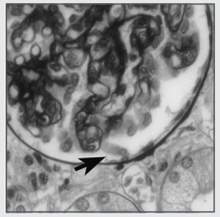
Figure S5



CD-44 positive cells on a podocyte location (full colour version inside cover)

Sections were co-stained for WT-1 (dark grey) and CD44 (green). Note the WT-1-positive cells indicating podocytes (A, an example is indicated by an arrow). In Panel B, a CD44-positive cell (arrow), is also WT-1 positive (A, arrow).

Figure S6



Cellular bridge (full colour version inside cover)

This figure shows an example of a cellular bridge (arrow) in a silver stain.

References

1. Steegers EA, von Dadelszen P, Duvekot JJ, Pijnenborg R: Pre-eclampsia. ***Lancet,*** 376**:** 631-644, 2010
2. Karumanchi SA, Maynard SE, Stillman IE, Epstein FH, Sukhatme VP: Preeclampsia: a renal perspective. ***Kidney Int,*** 67**:** 2101-2113, 2005
3. Lohlein M: Zur Pathogenese der Nierenkrankheiten; Nephritis und Nephrose mit besonderes Berücksichtigung der Nephropathia gravidarum. ***Deutsche Medizinische Wochenschrift***, 1918 pp 1187-1189
4. Vikse BE: Pre-eclampsia and the risk of kidney disease. ***Lancet,*** 382**:** 104-106, 2013
5. Garovic VD, Wagner SJ, Turner ST, Rosenthal DW, Watson WJ, Brost BC, Rose CH, Gavrilova L, Craigo P, Bailey KR, Achenbach J, Schiffer M, Grande JP: Urinary podocyte excretion as a marker for preeclampsia. ***Am J Obstet Gynecol,*** 196**:** 320-327, 2007
6. Kelder TP, Penning ME, Uh HW, Cohen D, Bloemenkamp KW, Bruijn JA, Scherjon SA, Baelde HJ: Quantitative polymerase chain reaction-based analysis of podocyturia is a feasible diagnostic tool in preeclampsia. ***Hypertension,*** 60**:** 1538-1544, 2012
7. Aita K, Etoh M, Hamada H, Yokoyama C, Takahashi A, Suzuki T, Hara M, Nagata M: Acute and transient podocyte loss and proteinuria in preeclampsia. ***Nephron Clin Pract,*** 112**:** c65-c70, 2009
8. Garovic VD, Wagner SJ, Petrovic LM, Gray CE, Hall P, Sugimoto H, Kalluri R, Grande JP: Glomerular expression of nephrin and synaptopodin, but not podocin, is decreased in kidney sections from women with preeclampsia. ***Nephrol Dial Transplant,*** 22**:** 1136-1143, 2007
9. Zhao S, Gu X, Groome LJ, Wang Y: Decreased nephrin and GLEPP-1, but increased VEGF, Flt-1, and nitrotyrosine, expressions in kidney tissue sections from women with preeclampsia. ***Reprod Sci,*** 16**:** 970-979, 2009
10. Casparie M, Tiebosch AT, Burger G, Blauwgeers H, van de Pol A, van Krieken JH, Meijer GA: Pathology databanking and biobanking in The Netherlands, a central role for PALGA, the nationwide histopathology and cytopathology data network and archive. ***Cell Oncol,*** 29**:** 19-24, 2007
11. Brown MA, Lindheimer MD, de SM, Van AA, Moutquin JM: The classification and diagnosis of the hypertensive disorders of pregnancy: statement from the International Society for the Study of Hypertension in Pregnancy (ISSHP). ***HypertensPregnancy,*** 20**:** IX-XIV, 2001
12. Schutte JM, Steegers EA, Schuitemaker NW, Santema JG, de BK, Pel M, Vermeulen G, Visser W, van RJ: Rise in maternal mortality in the Netherlands. ***Br J Obstet Gynaecol,*** 117**:** 399-406, 2010
13. Mundlos S, Pelletier J, Darveau A, Bachmann M, Winterpacht A, Zabel B: Nuclear localization of the protein encoded by the Wilms' tumor gene WT1 in embryonic and adult tissues. ***Development,*** 119**:** 1329-1341, 1993
14. Scholzen T, Gerdes J: The Ki-67 protein: from the known and the unknown. ***J of Cell Physiol,*** 182**:** 311-322, 2000
15. Strevens H, Wide-Swensson D, Hansen A, Horn T, Ingemarsson I, Larsen S, Willner J, Olsen S: Glomerular endotheliosis in normal pregnancy and pre-eclampsia. ***Br J Obstet Gynaecol,*** 110**:** 831-836, 2003
16. Lemley KV, Bertram JF, Nicholas SB, White K: Estimation of glomerular podocyte number: a selection of valid methods. ***J Am Soc Nephrol,*** 24**:** 1193-1202, 2013
17. Fatima H, Moeller MJ, Smeets B, Yang HC, D'Agati VD, Alpers CE, Fogo AB: Parietal epithelial cell activation marker in early recurrence of FSGS in the transplant. ***Clin J Am Soc Nephrol,*** 7**:** 1852-1858, 2012
18. Weibel ER, Gomez DM: A principle for counting tissue structures on random sections. ***J of Applied Physiol,*** 17**:** 343-348, 1962
19. Appel D, Kershaw DB, Smeets B, Yuan G, Fuss A, Frye B, Elger M, Kriz W, Floege J, Moeller MJ: Recruitment of podocytes from glomerular parietal epithelial cells. ***J Am Soc Nephrol,*** 20**:** 333-343, 2009
20. Craici IM, Wagner SJ, Bailey KR, Fitz-Gibbon PD, Wood-Wentz CM, Turner ST, Hayman SR, White WM, Brost BC, Rose CH, Grande JP, Garovic VD: Podocyturia predates proteinuria and clinical features of preeclampsia: longitudinal prospective study. ***Hypertension,*** 61**:** 1289-1296, 2013
21. Peired A, Angelotti ML, Ronconi E, la Marca G, Mazzinghi B, Sisti A, Lombardi D, Giocaliere E, Della Bona M, Villanelli F, Parente E, Ballerini L, Sagrinati C, Wanner N, Huber TB, Liapis H, Lazzeri E, Lasagni L, Romagnani P: Proteinuria impairs podocyte regeneration by sequestering retinoic Acid. ***J Am Soc Nephrol,*** 24**:** 1756-1768, 2013
22. Smeets B, Uhlig S, Fuss A, Mooren F, Wetzels JF, Floege J, Moeller MJ: Tracing the origin of glomerular extracapillary lesions from parietal epithelial cells. ***J Am Soc Nephrol,*** 20**:** 2604-2615, 2009
23. Smeets B, Kuppe C, Sicking EM, Fuss A, Jirak P, van Kuppevelt TH, Endlich K, Wetzels JF, Grone HJ, Floege J, Moeller MJ: Parietal epithelial cells participate in the formation of sclerotic lesions in focal segmental glomerulosclerosis. ***J Am Soc Nephrol,*** 22**:** 1262-1274, 2011
24. Chipps TJ, Appukuttan B, Pan Y, Smith JR: CD44 isoforms in human retinal and choroidal endothelial cells. ***Graefe's archive for clinical and experimental ophthalmology = Albrecht von Graefes Archiv fur klinische und experimentelle Ophthalmologie,*** 251**:** 1245-1246, 2013
25. Macconi D, Sangalli F, Bonomelli M, Conti S, Condorelli L, Gagliardini E, Remuzzi G, Remuzzi A: Podocyte repopulation contributes to regression of glomerular injury induced by ACE inhibition. ***Am J Pathol,*** 174**:** 797-807, 2009
26. Smeets B, Angelotti ML, Rizzo P, Dijkman H, Lazzeri E, Mooren F, Ballerini L, Parente E, Sagrinati C, Mazzinghi B, Ronconi E, Becherucci F, Benigni A, Steenbergen E, Lasagni L, Remuzzi G, Wetzels J, Romagnani P: Renal progenitor cells contribute to hyperplastic lesions of podocytopathies and crescentic glomerulonephritis. ***J Am Soc Npehrol,*** 20**:** 2593-2603, 2009
27. 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,*** 25**:** 3289-3296, 2010
28. Vikse BE, Irgens LM, Leivestad T, Skjaerven R, Iversen BM: Preeclampsia and the risk of end-stage renal disease. ***N Engl J Med,*** 359**:** 800-809, 2008
29. Eremina V, Jefferson JA, Kowalewska J, Hochster H, Haas M, Weisstuch J, Richardson C, Kopp JB, Kabir MG, Backx PH, Gerber HP, Ferrara N, Barisoni L, Alpers CE, Quaggin SE: VEGF inhibition and renal thrombotic microangiopathy. ***N Engl J Med,*** 358**:** 1129-1136, 2008
30. Sison K, Eremina V, Baelde H, Min W, Hirashima M, Fantus IG, Quaggin SE: Glomerular structure and function require paracrine, not autocrine, VEGF-VEGFR-2 signaling. ***J Am Soc Nephrol,*** 21**:** 1691-1701, 2010
31. Rana S, Karumanchi SA, Lindheimer MD: Angiogenic factors in diagnosis, management, and research in preeclampsia. ***Hypertension,*** 63**:** 198-202, 2014
32. Barisoni L, Schnaper HW, Kopp JB: Advances in the biology and genetics of the podocytopathies: implications for diagnosis and therapy. ***Arch Pathol Lab Med,*** 133**:** 201-216, 2009
33. D'Agati VD: Podocyte injury in focal segmental glomerulosclerosis: Lessons from animal models (a play in five acts). ***Kidney Int,*** 73**:** 399-406, 2008
34. Moeller MJ, Smeets B: Novel target in the treatment of RPGN: the activated parietal cell. ***Nephrol Dial Transplant,*** 28**:** 489-492, 2013

35. Benigni A, Morigi M, Rizzo P, Gagliardini E, Rota C, Abbate M, Ghezzi S, Remuzzi A, Remuzzi G: Inhibiting angiotensin-converting enzyme promotes renal repair by limiting progenitor cell proliferation and restoring the glomerular architecture. *Am J Pathol,* 179**:** 628-638, 2011
36. Lasagni L, Ballerini L, Angelotti ML, Parente E, Sagrinati C, Mazzinghi B, Peired A, Ronconi E, Becherucci F, Bani D, Gacci M, Carini M, Lazzeri E, Romagnani P: Notch activation differentially regulates renal progenitors proliferation and differentiation toward the podocyte lineage in glomerular disorders. *Stem Cells,* 28**:** 1674-1685, 2010