

# Proteinuria in Preeclampsia from a Podocyte Injury Perspective

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**Abstract** Preeclampsia is a frequent cause of maternal and fetal morbidity and mortality worldwide. The underlying causes of this hypertensive complication have remained elusive. The placenta seems to be at the origin of the disease, as its removal appears to be the only effective treatment available. Many organs can potentially be affected. Nonetheless, kidney alterations are always present: proteinuria is one of the hallmarks for a preeclampsia diagnosis. VEGF is pivotal for maintaining glomerular filtration barrier function; hence, the elevated concentrations of placental-derived VEGF inhibitors, such as sFlt-1, may largely explain the renal alterations observed. Classically, glomerular endothelial injury was considered responsible for the renal impairment present in preeclampsia. Recent findings, however, have shown that podocytes are crucial in explaining the loss of filtration capacity of the preeclamptic kidney. The aims of this manuscript are to detail the main findings that associate podocyte injury with proteinuria in preeclampsia, and discuss the eventual applications of podocyte damage biomarkers in clinical practice.

**Keywords** Podocyte · Proteinuria · Preeclampsia · Podocytopathy · VEGF · sFlt-1 · Hypertension

## Introduction

Preeclampsia is defined as the onset of hypertension and proteinuria that usually occurs after the 20th week of gestation [1]. Preeclampsia is a highly prevalent syndrome – approximately 6 % of all pregnancies worldwide are affected by this condition. The prognosis of preeclampsia is extremely variable: while the vast majority of women experience an adequate obstetrical outcome, some of them suffer adverse and severe complications such as eclampsia, HELLP (hemolysis, elevated liver enzymes, low platelet count) syndrome, and multi-systemic organic dysfunction that can frequently compromise the life of the mother and the fetus [2]. Several risk factors for the development of preeclampsia have been identified, but the aetiology of the disease has remained elusive. The placenta seems to be central to the underlying aetiology of preeclampsia, as its removal is the only effective treatment available. Recently, the association between preeclampsia and adverse cardiovascular events later in life has been demonstrated [3]. The real nature of this association remains unclear. Is preeclampsia a risk factor for cardiovascular events later in life, or do these women possess a genetic condition that predisposes them to suffer both?

Preeclampsia possesses two pathophysiological stages. The preclinical stage consists of an abnormal placentation process: the normal vascular remodelling of spiral arteries by trophoblasts does not occur [4]. The causes for this aberration are not fully understood. On the other hand, the infarcts observed in placental specimens from women with preeclampsia are likely due to the failure to carry out this remodelling process and may cause ischemia, similar to that observed when the spiral arteries are obliterated [5]. In response to this hypoxic environment, the ischemic placenta secretes soluble factors that are responsible for the systemic

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manifestations that characterize preeclampsia during the clinical stage. The brain (eclampsia) and the liver (HELLP syndrome) can be affected by the soluble factors in preeclamptic sera; nonetheless, the kidney appears to be especially sensitive to the effects of these factors. Thus, proteinuria is considered one of the hallmarks in making a diagnosis of preeclampsia.

The glomerular filtration barrier, which is the ultimate structure responsible for the filtration function of the kidney, possesses three components: the glomerular endothelium, the glomerular basement membrane and the podocytes. Podocytes are highly differentiated epithelial cells that form a complex molecular network known as the slit diaphragm. It is pivotal for maintaining the size-selective nature of the glomerular filtration barrier. The filtration function of the kidney is strongly associated with the formation and stability of the slit diaphragm. The abnormal expressions of slit diaphragm proteins, such as nephrin, podocin, CD2AP, and synaptopodin, seriously compromise the structure and function of the glomerulus [6]. Furthermore, such podocyte alterations have been related to severe proteinuric, congenital, and acquired conditions [7].

The kidney alterations in preeclampsia classically have been attributed to glomerular endothelial dysfunction [8]. Nonetheless, the recent evidence that associates proteinuria in preeclampsia with podocyte alterations has provided a novel perspective in the understanding of renal involvement in this disease. We aim to present in this review the recent findings that support this association and its eventual consequences for clinical practice.

### **Proteinuria in Preeclampsia: From the Glomerular Endothelium to Podocyte Injury**

Renal biopsies from women with preeclampsia show obliteration of endothelial fenestrae, endothelial edema, and obliteration of the capillary space [9]: a classical lesion known as glomerular endotheliosis (GEN). Interestingly, Strevens et al. [10] demonstrated that this lesion was present in women with preeclampsia and in women with gestational hypertension without proteinuria. Furthermore, they showed that GEN was also present in normal pregnant women. The presence of this glomerular lesion in patients with and without proteinuria may indicate that the mere endothelial lesion is not sufficient to explain the loss of filtration function in these women. As podocytes have been shown to be important for preventing proteinuria in other diseases, the possibility of studying podocyte injury in the context of preeclampsia is very attractive [11].

Garovic et al. [12] first demonstrated that slit diaphragm proteins from renal samples of women with preeclampsia

were significantly decreased when compared with samples from normal pregnant women. These descriptive findings indicated that podocytes are injured in preeclampsia, and that this molecular damage may be associated with proteinuria. This is contrary to evidence in electron micrographs of preeclamptic kidneys that show that the podocyte foot-process ultrastructures are relatively preserved [13]. A study by our group using a human podocyte cell line [14] demonstrated that the sera from women with preeclampsia directly affect podocyte function. The podocyte barrier-forming capacity is a dynamic measure of filtration function and we demonstrated that podocytes stimulated with preeclamptic sera have decreased barrier-forming capacity compared with normal pregnant women. Finally, evidence provided by Zhao et al. have corroborated that podocytes are structurally damaged in preeclamptic women [15].

These data clearly pointed towards podocyte involvement as a mechanism explaining proteinuria in preeclampsia. Collino et al. [16] first demonstrated a possible mechanism for this podocyte damage. The group was not able to prove a direct effect of preeclamptic sera on podocytes, but when podocytes were cultured with supernatant recovered from glomerular endothelial cells supplemented with preeclamptic sera, the podocytes demonstrated nephrin shedding. The podocyte damage was found to be mediated through the expression of Endothelin-1 by glomerular endothelium.

There appears to be an association between podocyte injury and proteinuria in preeclampsia, but whether podocyte damage is a cause or an effect of proteinuria in these women has yet to be determined. The answer may be determined ultimately from a better understanding of the inter-relationship between podocytes and the glomerular endothelium [17]. Vascular endothelial growth factor (VEGF) is fundamental for maintaining this relationship. How precisely can the high concentrations of VEGF inhibitors in the sera from women with preeclampsia affect this relationship?

### **From the Preeclamptic Placenta to the Kidney: The Role of Anti-VEGF Proteins on the Glomerulus**

VEGF has been recognized widely for its importance in vasculogenesis, wound healing, and tumour vascularization processes [18]. This last function is the target of many treatments that aim to control tumour invasion, with frequent treatment side effects of hypertension and proteinuria [19]. On the other hand, the high concentrations of VEGF inhibitors, mainly sFlt-1, in women with preeclampsia suggest the importance of VEGF in stabilizing mature blood vessels. Mahara et al. [20] described that the epithelial cells that overlie

fenestrated and sinusoidal blood vessels express VEGF, thus highlighting podocytes as the main source of VEGF within the glomerulus.

The importance of podocyte-derived VEGF was clearly demonstrated by Eremina et al. [21]. This group developed knockout mice specifically for podocyte-derived VEGF, with the mice exhibiting a severe glomerular phenotype consistent with podocyte injury. This study was seminal in demonstrating the exquisite dose dependent effects of VEGF in the glomerulus, wherein the complete podocyte specific knockout failed to develop endothelial cells, but the heterozygous (50 %) knockout developed endotheliosis and proteinuria similar to that seen in preeclampsia.

The diminished availability of VEGF in the preeclamptic glomerulus, due to high concentrations of sFlt-1, may alter podocytes by two different mechanisms [22]. The presence of sFlt-1 interrupts VEGF flow from the podocyte to the endothelium in an indirect manner. Injured endothelium produces endothelin-1 and its toxic effect on the podocyte is responsible for podocyte damage, and ultimately, proteinuria will occur. On the other hand, sFlt-1 can directly disrupt the autocrine loop for podocyte-derived VEGF. Recently, our group demonstrated [23••] that preeclamptic sera –with high sFlt-1 concentrations– can directly alter podocyte structure and function, and furthermore, that supplementing these sera with VEGF can directly reverse the altered podocyte barrier-forming capacity induced by preeclamptic sera alone. The biological plausibility for an autocrine loop has been proven both in vitro and in vivo. The podocyte expresses the neuropilin-1 receptor [24] that facilitates VEGF binding on the podocyte surface. Further evidence is needed to clearly determine the details of this paracrine/autocrine podocyte-derived VEGF; nonetheless, the molecule is important for podocyte survival [25] (Fig. 1).

Recent findings presented by Li et al. [26••] have demonstrated that reduced maternal endothelial nitric oxide synthase (eNOS)/nitric oxide exacerbate the sFlt1-related preeclampsia-like phenotype through activation of the endothelin system. This lends further evidence to support that the glomerular alterations present in patients with preeclampsia are due to the relative reductions in the levels of VEGF within the preeclamptic glomerulus (Fig. 1).

Podocytes are necessary to maintain the function of the glomerular filtration barrier. The glomerular endothelial cell is also a fundamental structural component of this barrier. Their functions depend on the availability of correct VEGF concentrations within the glomerulus. Thus, the high concentration of sFlt-1 present in sera from women with preeclampsia affects the communication between podocytes and glomerular endothelial cells, ultimately altering the functions of both cells. Nonetheless, if we accept that the

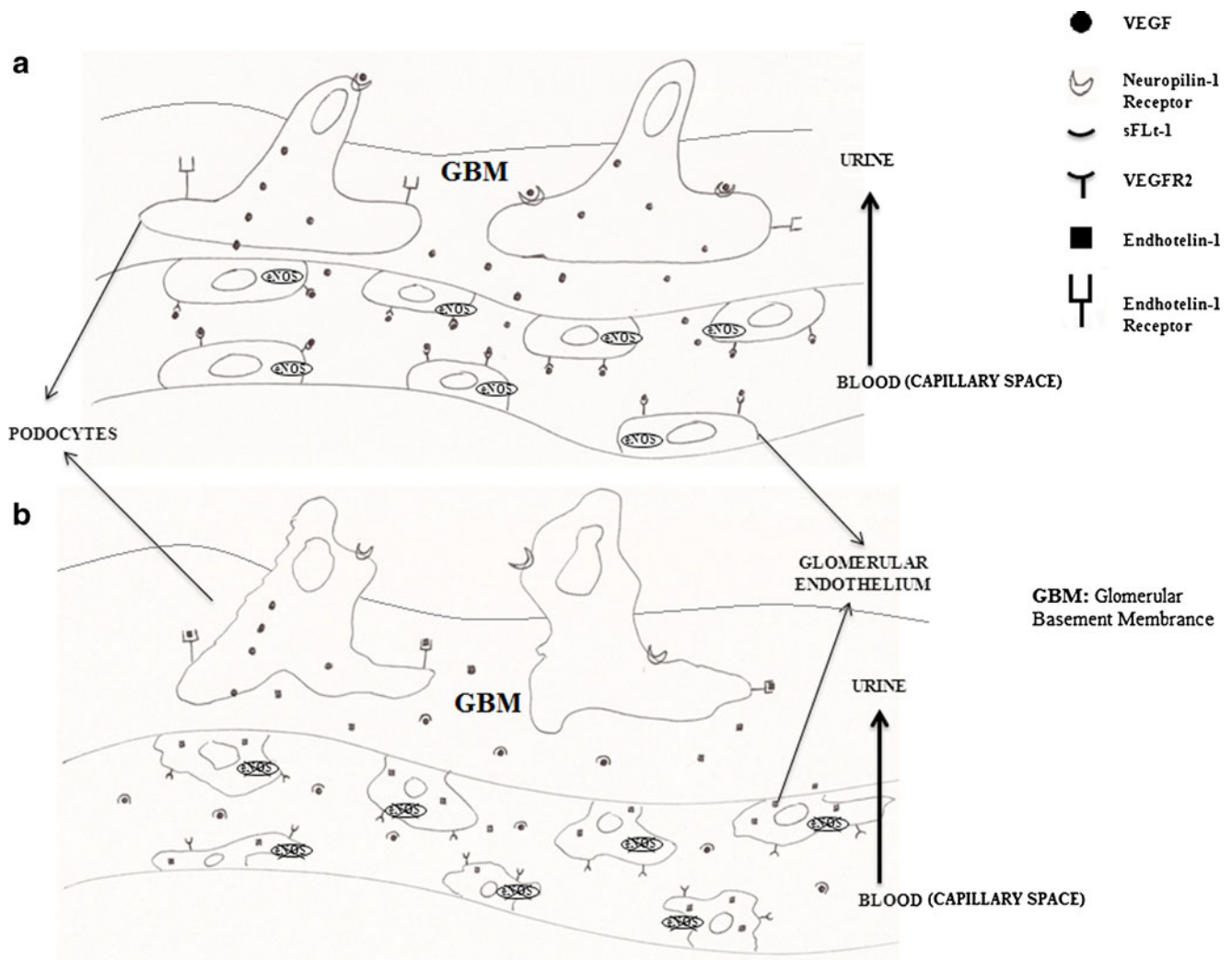
glomerular endothelial lesion is not sufficient to explain proteinuria, podocyte injury that might result from the blockage of the autocrine VEGF loop, or by the effect of a toxic mediator produced by the injured endothelium, could explain the presence of proteinuria in women with preeclampsia. The clinical utility of these data has not yet been firmly established.

### Can the Markers of Podocyte Injury Be Used in Daily Obstetrical Practice?

The lack of understanding as to the underlying causes of preeclampsia complicates the identification of precise markers for its diagnosis and prognosis. The urines of preeclamptic women have been studied for almost two centuries in the pursuit of a marker that can predict the outcomes of these patients. As podocyte injury has been identified as the possible lesion that results in the development of proteinuria, the search for clinically relevant markers of this damage has been intense. Garovic et al. [27] first demonstrated that podocytes are present in the urine (podocyturia) of preeclamptic women. This finding may be useful in the diagnosis of this condition, and these results are very promising, as podocyturia was present in all women with preeclampsia in their study. However, Jim et al. [28•] have suggested that podocyturia is not as sensitive or specific as previously shown, as only 11 of 29 preeclamptic women they studied presented with podocyturia. These discrepant results can be explained conceivably from a methodological perspective, as one group cultured overnight urinary samples, while the other group identified podocytes in urinary cytopins. In addition, the sensitivity and specificity of the podocyturia test may be affected by the choice of the podocyte-specific protein (nephrin vs. synaptopodin vs. podocin) used for the identification of urinary podocytes. Specifically, nephrin and synaptopodin may be down-regulated in preeclampsia by the disease process itself.

It is also not clear how podocyturia, resulting from preeclampsia, can be distinguished from podocyturia arising as a result of disease exacerbation of a concurrent but unrelated glomerulopathy. Further research will be needed to address this question. The recent evidence presented by Craici et al. [29••] begins to address this and suggests that podocyturia may be a very early marker for the development of pre-eclampsia.

Many groups are working to overcome the technical difficulties associated with the detection and processing of urinary podocytes. The classical technique requires the overnight incubation of podocytes and trained personnel to count stained cells. Kelder et al. [30••] have shown that



**Fig. 1** The figure represents a schematic model of the glomerular filtration barrier in healthy and pre-eclamptic women. Under normal circumstances, (A) podocyte derived VEGF acts on the podocytes – autocrine loop– and additionally, activates the VEGF receptor expressed on the glomerular endothelium –paracrine loop. On the podocytes, VEGF promotes its survival; on the glomerular, endothelium activates the eNOS and prevents the expression of Endothelin-1; thus the filtration function remains unaltered. On the other hand, in the pre-eclamptic glomerulus (B)

there is a high concentration of sFlt-1, derived from the ischemic placenta. This sFlt-1 binds and antagonizes VEGF. This diminished VEGF availability interrupts the autocrine VEGF loop on the podocytes and could prevent eNOS activation in the glomerular endothelium. Finally, the expression of Endothelin-1 within the glomerulus would affect the podocyte, i.e., producing nephrin shedding, and ultimately the occurrence of proteinuria

mRNA levels of slit diaphragm proteins (e.g., nephrin, podocin) are increased significantly in women with preeclampsia compared to healthy pregnant women, thus concluding that quantitative polymerase chain reaction analysis of podocyte-specific molecules in urine samples is a rapid method for measuring podocyturia. Podocalyxin is also another marker that may be of clinical utility [31•]. Finally, the mass spectrometry method to measure podocyte proteins in the urine recently proposed [32•] appears to be an interesting alternative.

Current evidence supports the biological plausibility of using podocyturia, as measured by the presence of podocyte-specific proteins, as a diagnostic and prognostic tool relevant for clinical practice. Nonetheless, further

epidemiological evidence is needed before incorporating it into daily practice.

**Conclusions**

Preeclampsia is a frequent and severe complication of pregnancy. Proteinuria is one of the hallmarks for its diagnosis. Although the glomerular endothelium had been considered the main structural component of the glomerular filtration barrier compromised in this disease, new evidence has suggested that podocyte injury may be equally responsible for the renal alterations. The main alterations related to podocyte damage are to the slit diaphragm proteins. Recent results demonstrating

that biomarkers of podocyte damage predate preeclampsia are encouraging, although further epidemiological research is needed to fully demonstrate their clinical utility.

### Compliance with Ethics Guidelines

**Conflict of Interest** Daniel E. Heno and Moin A. Saleem declare that they have no conflict of interest.

**Human and Animal Rights and Informed Consent** This article does not contain any studies with human or animal subjects performed by any of the authors.

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