GLomerular Epithelial Cells in the Urine:
What Has to Be Done to Make Them Worthwhile?

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Abstract

The significance of the native urine sediment in the differential of glomerular diseases needs no further comments. However the question arises whether it could be useful to develop a more specific diagnostic approach to identify the origin of renal epithelial cells that can be detected in the urine sediments as well. Especially the detection of podocytes in the urine could be a valuable non-invasive method to get information about the disease activity or disease type and could be used as a follow up after a biopsy in an outpatient setting. So far there are only a few studies that analyzed the clinical relevance of renal epithelial cells in the urine systematically or prospective. The reason for this could be the nature of the material since it will remain unclear whether detachment and changes in the urine milieu have a direct effect on the expression of marker proteins on the detected cells. Dedifferentiation or transdifferentiation of cells that goes along with changed marker expression is certainly also part of the underlying disease process. This review summarizes the available information on marker proteins that have been successfully used in the diagnostic of “podocytes” in the urine. Furthermore it gives an overview of marker expression on podocytes in situ in development and disease and examines the role of glomerular epithelial shedding in the urine at the interface of basic science and clinical medicine.
Introduction

Podocytes play an important role in the development and the maintenance of the glomerular tuft. They form a specialized structure—the slit diaphragm—, an electron dense membrane that spans the 30nm space between their interdigitating foot processes. The major component of the slit diaphragm is the transmembrane protein nephrin. In orchestra with a couple of other nephrin-interacting proteins they form a complex relevant for intracellular survival signalling and even mechanosensation. Changes in the glomerular micromilieu, expression of proinflammatory and proapoptotic cyto- and chemokines affect either the fate of the whole cell or lead to changes in the expression of slit diaphragm and other podocyte components. An imbalance of slit diaphragm components leads to foot process effacement and proteinuria. The proteinuria itself is a major stressor for glomerular and tubular cells. The loss of slit diaphragm components as well as the activation of TGF-β may lead to transdifferentiation, detachment and apoptosis. While the sequence of these events remains elusive, it is a widely accepted paradigm that podocytes are terminally differentiated epithelial cells with no potential for regenerative proliferation. There is evidence for specific expression of cell cycle related proteins in podocytes, however it remains controversial, whether this leads to a quantitatively relevant cellular division or whether that is simply a part of the disease process. This implies that if the cells are lost they cannot be replaced. Podocyte loss leads to a denuded basement membrane, adhesions, misfiltration of primary urine and in turn to inflammation and scarring. The role of podocytes in disease progression has been discussed in the context of many different rodent models as well as human glomerular diseases. Since podocyte loss seems to be an important component of disease progression in glomerular diseases it seems reasonable to look for podocytes in the urine as a possible marker of disease activity or adverse prognosis. One of the most robust marker that has been successfully used for urinary podocyte diagnostics is podocalyxin, a sialoprotein that is expressed on podocytes but also on a variety of non-renal as well as on glomerular endothelial and parietal epithelial cells. Other,
more podocyte specific marker proteins, especially slit diaphragm associated molecules were often inferior to podocalyxin in their diagnostic value. This review will focus on the presence or absence of marker proteins on glomerular epithelial cells in ontogeny and disease. Furthermore we will discuss the exciting prospect of utilizing urinary cell diagnostic to measure disease activity, degree of dedifferentiation or regeneration.

Markers for podocyturia diagnostics

Podocalyxin (PDX) - PDX is probably the most frequently used marker protein for podocyte diagnostic in the urine. It is a 140kDa sized polyanionic sialoprotein, localized in plasma membrane of podocytes (35). Due to its localization throughout the cell body podocalyxin remains well preserved in many glomerulopathies, independent of the integrity of the slit diaphragm. PDX has been used as a readout for podocyte excretion in many different renal diseases (i.e. membranous nephropathy (MN), membranoproliferative glomerulonephritis (MPGN), focal segmental glomerulosclerosis (FSGS), IgA nephropathy (IgAN), Henoch-Schoenlein Purpura (HSP) (21), lupus nephritis (LN), diabetic nephropathy (52) and preeclampsia (17)). In comparison to other markers PDX proved to be the most reproducible marker for urinary podocytes. According to Vogelmann et al., cells that stained positive for PDX, stained positive in 30–40% for other podocyte specific markers as well, while on the other hand cells positive for different podocyte specific markers stained always positive for PDX (79). It is important to note, that PDX expression is not podocyte specific. PDX is also present on endothelial cells, platelets, megakaryocytes, hemangioblasts and parietal epithelial cells (PECs) (see Table 1). While it has been shown that PDX-positive cells in the urine do not stain positive for endothelial and mesangial markers (32, 68, 87), the proportion of PECs among PDX-positive cells has not been fully elucidated (1). The use of PDX as a marker for urinary podocytes is further complicated by the fact that the amount of podocalyxin that is shed in the urine cannot originate solely from
podocytes (23). This discrepancy could be explained by PDX-shedding from stressed or apoptotic podocytes (79) and other PDX-positives cells i.e. PECs (1). Nevertheless assessing the total amount of podocalyxin or the total amount of podocalyxin positive cells in the urine, could be useful as a marker of disease activity. Determination of PDX-positive cells in the urine correlated with disease activity in patients with FSGS, MN, MPGN, IgAN and HSP (1, 21, 24). Measurements of PDX-protein by ELISA in the urine also correlated with histological changes and disease activity in children with IgAN, HSP, LN, MPGN and post-streptococcal glomerulonephritis (PSGN) (32) (Table 1). Several studies have also shown that the number of PDX-positive cells in the urine falls after various therapeutic interventions in patients with FSGS, LN, HSP, IgAN, PSGN and diabetic nephropathy (20, 47, 49-51, 53-55). A study done by Petermann et. al. on rats with different models of acute renal failure has shown that podocyturia normalizes much earlier than proteinuria, indicating that podocyturia is a more specific marker of ongoing acute renal injury than proteinuria (87). Of note is also the lack of PDX-positive cells in patients with minimal change disease, reported in several different studies, which might be clinically relevant as it could enable one to differentiate between MCD and FSGS in those cases where the histological diagnosis is questionable, perhaps due to poor sampling of glomeruli (1, 22, 53). There is also evidence that loss of PDX-positive cells, regardless of whether they represent podocytes or PECs, is linked to the development of glomerulosclerosis. A prospective study done by Hara et al. which examined patients with IgAN and HSP showed a strong correlation between the cumulative burden of the excretion of PDX-positive cells (the amount excreted in 6 consecutive months) and the development of glomerulosclerosis in follow-up biopsies (21).

**Nephrin and podocin.** Nephrin and podocin are both component proteins of the slit diaphragm-complex with a high degree of podocyte specificity (63). They play a critical role in maintaining the function of the glomerular filtration barrier. Both markers are not regularly
used for the staining of urinary cells. In the work of Garovic et al., podocin showed up as the most robust podocyte marker compared to PDX, nephrin and synaptopodin with 100% sensitivity in women with preeclampsia (17). Urinary presence of nephrin and podocin, assessed by RT-PCR correlated with the degree of proteinuria and the glomerular filtration rate (GFR) in diabetic nephropathy (82). Nephrinuria has been also presented as an early marker of developing renal complications in patients with type-1- diabetes (65). However, the staining of both of these proteins on podocytes is significantly reduced in proteinuric diseases (29, 56). Cultured podocytes exposed to nephrotic plasma (12) showed downregulation of nephrin and podocin. In contrast to that, expression of nephrin but not podocin was significantly reduced in renal tissue of women with preeclampsia (16). Taken together nephrin and podocin might be highly podocyte specific, however they seem highly regulated in proteinuric states, which is not surprising since foot process effacement and a reduction in intact glomerular filtration slits is a common feature of proteinuric renal diseases.

**Synaptopodin.** Synaptopodin is a podocyte specific, actin-cytoskeleton associated protein, which has several described functions (2). However expression can also be found in telencephalic dendrites (44). Similar to nephrin and podocin, synaptopodin expression on podocytes has been found to be diminished in renal biopsies of patients with various renal diseases (74). Podocytes react with a significant downregulation of synaptopodin when exposed to nephrotic plasma (12). Nonetheless, expression of synaptopodin measured by RT-PCR has been used for urinary podocyte identification in children with HUS (13). The usefulness of this marker has been questioned by Ihmoda et al., who demonstrated that detection of urinary podocytes using anti-synaptopodin antibodies in 100 proteinuric patients led to a positive result in only 1 case (30). Interestingly, in a rodent model (PHN rats) the urinary sediments were synaptopodin positive, but negative for podocin. However, mRNA measurements of other podocyte specific markers were positive as well (68). In cultured
murine podocytes synaptopodin is often used as a marker of podocyte differentiation. The immortalized podocytes become only positive after a 7 to 10 day differentiation period in non-permissive conditions. Therefore, synaptopodin might be a very sensitive marker for intact podocytes that is lost early on in disease states that involve cellular trans- or de-differentiation. The usefulness of synaptopodin in urine diagnostic therefore remains controversial.

**GLEPP1.** Glomerular epithelial protein (GLEPP1) is a membrane bound protein-tyrosine phosphatase present on the apical surface of podocytes, which plays a role in regulating the structure and function of the foot processes (76). It appears quite podocyte specific although its expression has been also detected in the brain (76). Various articles used GLEPP1 to identify urinary podocytes but there is no work where it was applied as a single marker. GLEPP1 expression has been demonstrated to be well preserved in MCD and MN, whereas a significant reduction has been noted in collapsing forms of FSGS and IgA nephropathy (3, 9, 77, 85).

**Wilms tumor protein (WT-1)**

Wilms tumor protein (WT1) is characteristic for adult podocytes. It is a zinc finger protein which regulates the transcription of PDX (64), nephrin (80) and PAX-2 (70). WT1 is well preserved in MCD and MN but in collapsing form of FSGS it is strongly downregulated (9). WT1-positive cells were found in the urine in PHN rats (68), PAN rats, anti-Thy 1 nephritis and 5/6-nephrectomy rat model (87). Using RT-PCR technique urinary WT1 mRNA correlated significantly with the degree of tubulointerstitial fibrosis in diabetic nephropathy (82). Interestingly, contrary to these findings *Vogelmann et al.* stated that WT1 staining was negative, although all other podocyte markers (i.e. podocalyxin, podocin, synaptopodin and GLEPP1) were present in their urinary cultures (79).
From the above described variety of podocyte markers it becomes quite clear that enough specific marker proteins for glomerular epithelial diagnostics are available, however we have no certainty how these marker proteins might be influenced by the disease process itself or whether changes might occur after the cells are detached, pass the tubules and collecting ducts until we finally can analyze them. Therefore it seems important to give an overview on the known developmental and disease associated changes in marker expression.

Marker expression on glomerular epithelial cells in situ in development and disease.

In Table 2 we summarized the marker expression on visceral podocytes, parietal podocytes or parietal epithelial cells (PEC), in normal adult or fetal kidneys. The staining characteristics of normal mature visceral podocytes (VP) have been extensively described in various studies (3, 5-7, 9, 10, 37, 46, 85, 86). Only recently Bariety and coworkers defined the immunohistochemical characteristics of so called parietal podocytes (PP) (7). PPs are epithelial cells that adhere to Bowman’s capsule mainly at the vascular pole and are morphologically identical to VPs (62). PPs express the same markers as VPs with some important differences. They stain less frequently for p57, a cyclin-dependent kinase (CDK) inhibitor, which prevents cellular division by blocking the CDK complexes (72). This is contrary to normal mature VPs which are regularly positive for p57 (5, 7, 10). The p57 positivity of VPs is nicely in line with the current paradigm that visceral podocytes are terminally differentiated cells incapable of postnatal division (11). Furthermore PPs coexpress WT1 and PAX-2, two transcription factors with important roles in nephrogenesis. The PAX-2 gene, a mammalian homeobox gene, encodes for a transcription factor that is essential for the conversion of cells of the metanephric mesenchyme to the renal vesicle. PAX-2 plays a crucial role in early glomerular development, when most of the cell proliferation of glomerular epithelial cells occurs (27, 67). It is subsequently lost from VPs, usually at the
capillary loop stage (7) and is “replaced” by WT1, which is already present in VPs at the earliest stages of nephrogenesis (9, 67). WT1 is the predominant nuclear transcription from the capillary loop stage onwards. This downregulation of PAX-2 by WT1 is thought to be the pivotal event in the final differentiation of podocytes into a terminally differentiated cell (9, 67). The fact that parietal podocytes exhibit diminished staining of p57 and express PAX-2 might indicate that these cells have retained the ability to divide (7).

The staining characteristics of normal mature PECs have also been well described in various studies (Table 2). The markers that are most often used to identify PECs are cytokeratins, which are present in normal PEC (3, 5, 7, 19, 26, 37, 41, 86) and are upregulated in disease states such as FSGS (14). Cytokeratins are also the most predominantly positive marker expressed by hyperplastic proliferating epithelial cells that are found in collapsing FSGS forms and HIV associated nephropathy (HIVAN) (14, 15, 37, 39, 45, 46, 59) (Table 3). It has been proposed by Barisoni et al. (9) and others (3, 59, 85), that these cells are actually dedifferentiated podocytes that lose their characteristic markers and gain the expression of cytokeratins, which are normally never present on podocytes (3, 5, 7, 37, 46, 86) (Table 2).

There is some evidence to support this hypothesis. It has often been shown that podocytes in some disease states undergo a dedifferentiation, exemplified by the loss of podocyte related markers, like podocalyxin, synaptopodin and GLEPP-1 (5, 15, 34, 74). Studies in crescentic GN have shown normal looking VPs in non-affected glomeruli expressing cytokeratins (5, 6). Cytokeratins are also positive in immature podocytes during nephrogenesis (7). Immature looking podocytes with cobblestone appearance have also been demonstrated to express cytokeratins in certain children with MPGN (62). Two studies have shown cells co-expressing podocalyxin and cytokeratin in posttransplantation FSGS (3) and collapsing FSGS (45), which could be interpreted as a sign of podocyte dedifferentiation.

Another feature of the hyperplastic proliferating epithelial cells in FSGS and HIVAN is that they are WT1 negative (Table 2). This seems to controvert the dedifferentiated podocyte
hypothesis since podocytes express WT1 in all stages development (7, 9, 67). However mature PECs do not express WT1 (9), so it seems possible that PECs could be the origin of hyperplastic proliferating cells in FSGS. Furthermore, normal PECs often express PAX-2 (7) and at times also Ki-67 (5, 45, 46), a well known proliferation marker, indicating their innate proliferative capability. Dijkman et al. and Nagata et al. have provided further evidence to support this hypothesis, as they have shown that cytokeratin positive hyperplastic cells are in continuity with PECs on Bowman’s capsule in FSGS including the collapsing variant, either idiopathic or associated with HIV infection or pamidronate use (14, 15, 45). In addition Dijkman et al. found synaptopodin positive podocytes covering the collapsed glomerular tufts, which were in turn covered by synaptopodin negative but cytokeratin and PAX-2 positive epithelial cells (14). Importantly there were no cells coexpressing synaptopodin and PAX-2, VEGF (as a podocyte marker) and cytokeratin (14) or synaptopodin and cytokeratin (15), making it less likely that these cells are dedifferentiated podocytes. They have also shown that the extracellular matrix deposited in the lesions was collagen IVα1 and heparan sulphate single chain HS4E4 positive (a characteristic component of the parietal basal membrane). However it was negative for collagen IVα3 and heparan sulphate single chain HS4C3 (a characteristic component of the glomerular basement membrane) (14, 15). Furthermore, Nagata et al. examined the ultrastructural characteristics of hyperplastic cells in FSGS with electron microscopy. They describe that these cells are frequently located on the parietal side. They have junctional complexes with neighbouring hyperplastic cells and some of these cells have a cellular base on the Bowman’s capsule. Additionally these cells exhibit cilia, which would indicate that they are actually PECs (46).

These data indicate how difficult it can be to determine the origin of glomerular epithelial cells in disease states based solely on information from immunohistochemical staining.

Another point of ongoing discussion is the origin of proliferating cells in crescents. The majority of cells in crescents have been shown to stain for different epithelial markers (e.g.
cytokeratin, PGP9.5, Pan cadherin) indicating that PECs are the predominant cell type in crescents (73, 78) (Table 3). Cells exhibiting macrophage related markers, especially CD68, have also been implicated in the formation of crescents (4, 5, 31, 42, 57, 84), although most studies have reported that these cells represented a minority (31, 84). Findings by Bariety et al. (3), describing cells coexpressing podocalyxin or cytokeratin and CD68 support the hypothesis that glomerular epithelial cells are the origin of these macrophage-like cells. However these findings were not confirmed in other studies (6). This epithelial transdifferentiation has also been described in patients with idiopathic collapsing glomerulopathy (8). In advanced fibro-cellular and fibrous crescents α-smooth muscle actin expressing myofibroblasts have an important pathogenetic role (5, 18). These cells could also originate via epithelial transdifferentiation, since cells that coexpress α-SMA and cytokeratin have been found in crescentic GN (4, 6). However, they could also represent myofibroblasts originating from the periglomerular interstitium (18).

A variety of papers have shown that the majority of cells in crescents do not stain for any other marker except vimentin (4-6) (Table 3). Vimentin is an intermediate filament that is known to be present on VPs and PPs (3, 5, 7, 85, 86), indicating that podocytes might be involved in the formation of crescents. A recently published study has shown that a low percentage of cells in crescents expresses different podocyte related markers (podocalyxin, GLEPP-1, podocin, synaptopodin, α-actinin4) (5). These findings would further support the role of podocytes in crescentic GN. Given the low number of cells exhibiting podocyte markers in crescents it also seems possible that these cells are simply PPs “trapped” in the forming crescent. The fact that the majority of cells are vimentin positive does not exclude PECs as a possible origin of proliferating cells. Bariety et al. have shown in 13.3% of examined glomeruli from normal adult human kidneys vimentin positivity along the whole circumference of Bowman’s capsule. This could not be attributed to parietal podocytes since only 2.6% of examined glomeruli exhibited podocalyxin positivity along whole
circumference. The percentage was even lower for other podocyte related markers. As a consequence a certain proportion of parietal epithelial cells have to be vimentin positive as well (7). Vimentin positive PECs have been documented by other authors as well (5, 57), therefore the traditional conception that PECs are vimentin negative might be misleading. Progenitor cells lining the Bowman’s capsule with stem cell characteristics could also account for proliferating cells. These cells where first identified by Sagrinati et al. with positive anti-CD24 and anti-CD133 staining and dominate the part of the Bowman’s capsule adjacent to the urinary pole (71). These cells co-stained also for cytokeratin and vimentin positive. It therefore remains challenging to determine the exact origin of proliferating cells in crescentic GN by immunohistochemical techniques.

As previously discussed, the vast majority of studies determining podocytes in the urine used podocalyxin as a readout for podocyte loss in the urine. However contrary to published staining characteristics of PECs in normal kidneys with no expression of podocalyxin (3, 37, 46) (Table 2), we found in our study that PECs were invariably podocalyxin positive in patients with FSGS, membranous nephropathy and minimal change disease (1), a finding that has previously already been reported in FSGS patients (3). This discrepancy could be due to the fact, that PECs might be dedifferentiated in certain disease states. During nephrogenesis PECs express podocalyxin and GLEPP-1 up to the capillary loop stage and only lose these markers in the final stages of glomerular development (3). There is evidence from another study that PECs can undergo dedifferentiation, demonstrating that normal PECs express caveolin-1, while PECs in patients with FSGS or lupus nephritis do not (61). However we attribute these seemingly contradictory results to technical issues. In our view podocalyxin is always present on PECs, however in a very low concentration compared to podocytes. In a serial dilution series we were able to stain podocalyxin on PECs in kidney samples of normal kidneys (personal observation). The PECs are positive for podocalyxin using the same antibody concentration necessary to stain endothelial cells, which are known to be
podocalyxin positive. Therefore one should be aware that differences in staining techniques, tissue handling and fixation might have influenced the quality, results and interpretation of all the above cited articles.

**Future directions**

We summarized above the published evidence how investigators performed specific cell diagnostics in urine. To avoid pitfalls it is important to define the questions one would like to answer beforehand. The question will dictate the approach one should take in specific urinary cell type diagnostics. Determining the amount of mature differentiated podocytes in the urine is certainly highly significant, since this could be interpreted as irreversible glomerular damage. This however turns out to be the most difficult approach since using any single podocyte related marker for determining urinary podocyte loss seems unreliable. Most of the well recognized podocyte specific markers are either lost in different stages of glomerular diseases or can also be detected in PECs (Table 2 and 3). Podocalyxin staining has been the method of choice in the vast majority of published articles, however it has been discovered that PECs also express podocalyxin in various disease states. Therefore, detecting podocalyxin positive cells in urine might as well be a consequence of PEC loss. Given the current available information it seems that the most reliable way to determine podocytes in the urine would be a costaining for PDX and either WT1 or p57, since both of these markers seem to be present on podocytes even in disease states, while they are not traditionally associated with PECs (Table 2 and 3, Figure 1). However, the whole argument on whether these cells are podocytes or PECs might be clinically irrelevant. It might be enough to determine the extent of glomerular epithelial shedding into the urine to get an impression of ongoing disease activity. The amount of podocalyxin positive cells (which includes then VPs, PPs and PECs) correlates well with disease activity in various glomerular diseases, especially FSGS, membranous nephropathy and IgA nephropathy. Therefore, determining podocalyxin
positive cells in the urine using cytospins might still be a valid tool in estimating glomerular disease activity in these diseases. The method of culturing the urine sediments leads to a selection of viable cells that attach to collagen. In this case, it might be even unnecessary to stain the cells with a specific marker. In our study we found that the amount of podocalyxin positive cells correlated well with the total amount of cultured cells quantified by DAPI (1). Another interesting question that could be answered with urinary epithelial cell excretion is the extent of glomerular epithelial dedifferentiation. Doublestaining for cytokeratins and PAX-2 could reveal the number of dedifferentiated glomerular cells present in certain forms of FSGS (Table 3). While cytokeratins are commonly positive on tubular epithelial cells and PECs, PAX-2 is predominantly expressed early in nephrogenesis and is downregulated in mature tubular epithelial cells and podocytes. Therefore, PAX-2 could be used as a marker of dedifferentiation. However, one study observed PAX-2 expression in normal PECs as well (7). Staining for CD68 could be another approach to monitor disease activity. CD68 positive cells have been found in the glomeruli, in intratubular lumens and in the urine of patients with FSGS and proliferative GN (3, 5, 58). The CD68 cell count increases in the urine of patients with focal segmental glomerular sclerosis, but their numbers are negligible in minimal change nephrotic syndrome (58), making it a possible marker to differentiate between these two diseases. However again it remains unclear which cell type we are measuring with CD68, since macrophages as well as “transdifferentiated podocytes” stain positive for this marker.

Staining for vimentin positive cells could have clinical relevance in crescentic GN. Most of the cells in crescents do not stain for any marker except for vimentin. The number of vimentin positive cells in urine might represent an estimate of the amount of extracapillary proliferation, a known factor of adverse prognosis in these diseases. Changes in the number of vimentin positive cells could perhaps be useful as a follow up marker to monitor treatment success or disease relapse.
What is the clinical relevance?

The question whether marker expression on urinary cells is a solid and reliable readout of disease activity remains so far unanswered, since larger clinical studies and prospective studies on urinary epithelial cell diagnostic are rare. Independent of the techniques used it is absolutely clear that these types of measurement can never replace a biopsy. However, an excretion of glomerular epithelial cells might indicate destructive glomerular disease and therefore it could be helpful in decision making when to take a biopsy or when it is justifiable to wait for a treatment response in children with FSGS or MCD. Furthermore, we have no good marker of disease activity in membranous nephropathy. Proteinuria is certainly not a completely reliable readout of ongoing disease and the decision on immunosuppressive regimens in membranous nephropathy is often dependent on the nephrologists experience and the overall course of treatment response and kidney function in the individual case. In these patients the measurement of glomerular cell excretion could be a very helpful tool even in an outpatient setting. Another advantage is that urine analysis is a read-out of all functional nephrons in both kidneys, whereas the biopsy result could be afflicted with the risk of sampling error.

However, for the urine analysis it can be technically challenging to get a reproducible and reliable setup working. These techniques are often time consuming and require a special trained person. Thus, the readout of the assay could be observer dependent. Therefore future research has to focus on development of observer independent techniques that allow same-day results. Unfortunately, these assay types remain to be established.

In summary, detection of PDX or CD68 positive cells in urine could be a valuable tool in assessing disease activity of certain glomerular diseases. Especially in FSGS and membranous nephropathy these markers could be a very useful tool as a maker of disease activity even in an outpatient setting. So far, expression of marker proteins does not allow a definite allocation
of the involved glomerular cell types. Therefore the mechanistic interpretation of previously published studies on the topic has to be very cautious. Further research is needed and the development of innovative tools is necessary to determine the role of markers for urinary cell diagnostics in treating patients with glomerular diseases. Only with fast and observer-independent tools, prospective, multicenter-studies can be done to validate the usefulness of epithelial cell diagnostics in patients with glomerular disease.

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Figure legends

Figure 1: Schematic overview of marker expression during glomerular maturation and disease in visceral epithelial cells (VECs), parietal podocytes (PPs), parietal epithelial cells (PECs) and cells in crescents (CCs). Key steps during glomerular development is the switch of WT1 and cytokeratin expression between the S-shaped stage and the capillary loop stage which allows a differentiation between VEC/PP and PEC. The major difference of VECs and PPs is coexpression of WT1 and PAX2 in PPs whereas VECs are PAX2 negative. The marker panels in the mature stage clearly show (with markers used in published studies for podocyturia diagnostics highlighted in blue) that in most of the cases the use of a single marker protein allows no differentiation in PECs, VECs or PPs since the markers can be expressed in all compartments. This is especially important since marker expression might change with disease activity (see tables). Therefore if these markers are used as single identifiers for urine diagnostics one should just specify them as 'glomerular epithelial cells'.
## Tables

Table 1. Podocyte markers used in urinary cell diagnostics.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Cell type</th>
<th>Disease</th>
<th>Literature</th>
<th>Specific context</th>
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<td>Podocytes</td>
<td>IgA nephropathy</td>
<td>(60), (24), (32), (23),</td>
<td>Not podocytes specific: found also on platelets, megakaryocytes (43), endothelial cells (35), blood vesels (28), aorta-gonad-mesonephros (AGM) region of mouse embryos (25), haematopoietic stem cells (36), blasts of acute myeloid leukemia and acute lymphoblastic leukemia (33), Parietal epithelia cells (1)</td>
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<td>Schonlein-Hoenoch purpura</td>
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<td>Diabetic nephropathy</td>
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<td>Lupus nephritis</td>
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<td>FSGS</td>
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<td>preeclampsia</td>
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<td>Nephrin</td>
<td>Podocytes</td>
<td>preeclampsia</td>
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<td>Expressed on beta pancreatic cells (63), (88)</td>
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<td>passive Heymann nephritis (PHN) model</td>
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<td>HUS</td>
<td>(13) mRNA</td>
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<td>Lupus nephritis</td>
<td>(83) mRNA</td>
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<td>IgA nephropathy, MC, MN</td>
<td>(75) mRNA</td>
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<td>Podocytes</td>
<td>passive Heymann nephritis (PHN) model</td>
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<tr>
<td><strong>Normal mature human visceral podocytes</strong></td>
<td>podocalyxin ++ (3, 7, 9, 37), +++ (5) GLEPP-1 ++ (3, 7, 9), +++ (5, 85) podocin ++ (7), +++ (5) nephrin ++ (7) synaptopodin ++ (3, 7, 9, 37), +++ (5, 85) α-actinin-4 ++ (7), +++ (5) VEGF ++ (7) WT1 +++ (7, 85), ++ (3, 9) p57 +++ (5, 7, 10) vimentin +++ (5, 7), ++ (3, 85), N.A.</td>
<td>PAX2 (7, 85) cytokeratin (3, 5, 7, 37, 46, 86) PCNA (3, 5, 6, 85) Ki-67 (5, 9, 10) CD68 (3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage Description</td>
<td>Markers (and References)</td>
<td>Other Markers (and References)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------------------------</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Visceral podocytes during embryogenesis – capillary loop stage</td>
<td>podocalyxin ++ (3, 7, 9), N.A. (67) GLEPP-1 ++ (3, 7, 9), N.A. (67) synaptopodin ++ (3, 7, 9, 10), N.A. (67) podocin ++ (7), N.A. (67) nephrin N.A. (67) CD2AP N.A. (67) vimentin N.A. (67) WT1 ++ (7, 67) p57 ++ (7, 10) CR1 ++ (9) CALLA ++ (9) VEGF N.A. (67) Ki-67 +/- (9) cytokeratin +/- (7)</td>
<td>PAX2 (7) CD68 (3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visceral podocytes during embryogenesis – S shaped body</td>
<td>podocalyxin ++ (3, 7, 9), N.A. (67) GLEPP-1 ++ (3, 7, 9) synaptopodin ++ (7) podocin ++ (7) WT1 ++ (7, 67) p57 ++ (7) Ki-67 ++ (9, 10) CR1 ++ (9) cytokeratin N.A. (7) VEGF N.A. (67) PAX2 +/- (7)</td>
<td>synaptopodin (3, 9, 10, 67) CALLA (9) WT1 (3) cytokeratin (3) CD68 (3) p57 (10) GLEPP-1 (67) podocin (67) nephrin (67) CD2AP (67) vimentin (67)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visceral podocytes during embryogenesis – vesicle stage</td>
<td>Ki-67 ++ (9) WT1 ++ (9, 67) PAX-2 N.A. (27, 67)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal mature human parietal podocytes</td>
<td>podocalyxin ++ (7), + (5) GLEPP-1 ++ (7), + (5) podocin ++ (7), + (5) nephrin ++ (7) synaptopodin ++ (7), + (5) α-actinin-4 ++ (7), + (5) VEGF ++ (7) WT1 ++ (7) PAX2 ++ (coexpression with WT1) (7) p57 + (7) vimentin + (5, 7), +/- (3)</td>
<td>cytokeratin (7) CD68 (3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parietal podocytes during embryogenesis – capillary loop stage</td>
<td>podocalyxin ++ (7) GLEPP-1 ++ (7) synaptopodin ++ (7) podocin ++ (7) WT1 ++ (7) p57 ++ (7)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| **Parietal podocytes during embryogenesis – S shaped body** | PAX2 ++ (7)  
| cyto keratin + (7)  |
| podocalyxin ++ (7)  
| GLEPP-1 ++ (7)  
| synaptopodin ++ (7)  
| podocin ++ (7)  
| WT1 ++ (7)  
| p57 ++ (7)  
| PAX2 ++ (7)  
| cyto keratin + (7)  |
| **Parietal podocytes during embryogenesis – vesicle stage** | No available information |
| **Normal mature human parietal epithelial cells** | PAX2 ++ (7), N.A. (85)  
| cyto keratin ++ (3, 5, 37, 46) or + (26, 41) or +/- (7, 86), N.A. (19)  
| GLEPP-1 + (3)  
| PCNA +/- (5, 6)  
| Ki-67 +/- (5, 46)  
| p57 +/- (5)  
| vimentin + (7), +/- (5), N.A. (86)  
| WT1 N.A. (85)  |
| **Parietal epithelial cells during embryogenesis – capillary loop stage** | podocalyxin ++ (3)  
| GLEPP-1 ++ (3)  
| cytokeratin +/- (3)  |
| **Parietal epithelial cells during embryogenesis – S shaped body** | podocalyxin ++ (3)  
| GLEPP-1 ++ (3)  
| WT1 ++ (9)  
| cytokeratin +/- (3)  |
| **Parietal epithelial cells during embryogenesis – vesicle stage** | WT1 ++ (9)  |
| **Adult human parietal epithelial multipotent progenitor cell** | CD24 +++ (71)  
| CD133 +++ (71)  
| CD44 +++ (71)  
| CD54 +++ (71)  
| CD105 +++ (71)  
| CD106 +++ (71)  
| vimentin +++ (71)  
| cytokeratin +++ (71)  |

§ – reported as no examined cells being positive while staining with the given marker

*Degree of positive staining:*

+++ – reported as all examined cells positive  
++ – reported either as positive in most examined cells or the context implied that the staining in examined cells was predominantly positive, or when percentages are given ≥50% of examined cells positive  
+ – reported as some positive cells, or when percentages are given ≥10% but <50% of examined cells positive  
+/− – reported either as rare or few positive cells or the context implied that positive cells where a rare event, or when percentages are given <10% of examined cells positive
N.A. – examined cells were stained but quantitative data on the number of positive cells is not given

Table 3. Staining characteristics of glomerular epithelial cells in glomerular diseases.

<table>
<thead>
<tr>
<th>Cell type and specific context</th>
<th>Markers present *</th>
<th>Markers absent §</th>
</tr>
</thead>
</table>
| **Cells in glomerular crescents** | vimentin ++ (4, 5, 86) or + (86), N.A. (31)  
HSP47 ++ (6)  
cytokeratin ++ (19, 31, 41, 57, 78), + (4, 5, 19, 26, 41, 86), +/- (19, 41)  
α-actinin-4 + (5)  
Ki-67 + (57)  
synaptopodin + or +/- (5)  
GLEPP-1 + or +/- (5)  
podocin + or +/- (5)  
podocalyxin + (5) or +/- (5, 57)  
PCNA + (4-6), +/- (5, 6)  
CD68 + (4, 5) or +/- (5, 57)  
α-SMA + (5), +/- (4, 5) | p57 (5, 6)  
p27 (6, 57)  
podocalyxin (6)  
synaptopodin (6)  
GLEPP-1 (6)  
CR1 (31) |
| **Dedifferentiated glomerular epithelial cells in FSGS**  
(proliferating cells and/or cells exhibiting a cobblestone appearance) | cytokeratin ++ (14, 15, 37, 39, 45, 46, 59), + or +/- (3)  
CD68 +/- (45), N.A. (3, 37)  
PAX-2 +++ (15) or ++ (14, 39, 59), + (85)  
Ki-67 ++ (46), + (9, 10, 15, 45)  
vimentin +/- (85)  
PCNA +/- (85) | podocalyxin (3, 8, 9, 37, 45, 46)  
synaptopodin (3, 9, 10, 14, 15, 37, 39, 46, 85)  
GLEPP-1 (3, 9, 85)  
CD68 (8)  
WT1 (3, 9, 85)  
vimentin (3, 8, 14)  
CR1 (3, 8, 9)  
VEGF (14, 15, 39)  
CALLA (9, 14)  
p57 (10) |
| **FSGS – podocytes (not proliferating or morphologically dedifferentiated (cobblestone appearance), usually in normal looking glomeruli)** | podocalyxin +++ (1) ++ (3) N.A. (8, 37, 45)  
cytokeratin ++ (3)  
synaptopodin ++ (3) N.A. (14, 15, 37)  
GLEPP-1 ++ (3)  
WT1 ++ (3, 59)  
vimentin ++ (3) N.A. (8)  
CR1 ++ (3) N.A. (8)  
VEGF + (15)  
CALLA + (15) | cytokeratin (1, 45, 59)  
PAX-2 (59) |
| **FSGS – parietal epithelial cells** | podocalyxin +++ (1), +/- (3)  
cytokeratin ++ (45), + (1, 3, 14, 15, | synaptopodin (3, 14)  
CD10 (14) |
| **Membranoproliferative glomerulonephritis** – podocytes | podocalyxin +++ (1) | cytokeratin (1) |
| Membranoproliferative glomerulonephritis – parietal epithelial cells | podocalyxin +++ (1) | cytokeratin + (1) |
| **Membranous nephropathy** – podocytes | podocalyxin +++ (1), ++ (9) | cytokeratin (1) | Ki-67 (9) |
| | GLEPP-1 ++ (9, 85) | | PAX2 (85) |
| | synaptopodin ++ (9, 85) | | |
| | WT1 ++ (9) | | |
| | CR1 ++ (9) | | |
| | CALLA ++ (9) | | |
| | vimentin ++ (85) | | |
| Membranous nephropathy – parietal epithelial cells | podocalyxin +++ (1) | cytokeratin + (1) | PAX2 N.A. (85) |
| **Minimal change disease** – visceral podocytes | podocalyxin +++ (5), ++ (3, 9) | PCNA (5) | |
| | GLEPP-1 +++ (5), ++ (3, 9, 85) | Ki-67 (5, 9) | |
| | podocin +++ (5) | | |
| | synaptopodin +++ (5), ++ (3, 9, 85) | | |
| | α-actinin-4 +++ (5) | | |
| | vimentin +++ (5), ++ (3, 85) | | |
| | WT1 ++ (3, 9) | | |
| | CR1 ++ (3, 9) | | |
| | p57 +++ (5) | | |
| | CALLA ++ (9) | | |
| Minimal change disease – parietal podocytes | podocalyxin + (5) | | |
| | GLEPP-1 + (5) | | |
| | podocin + (5) | | |
| | synaptopodin + (5) | | |
| | α-actinin-4 + (5) | | |
| | vimentin + (5) | | |
| | p57 +++ (5) | | |

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N.A. – examined cells were stained but quantitative data on the number of positive cells is not given

References:


S-shaped stage

Podocalyxin
GLEPP1
WT1
Cytokeratin (+/-)

WT1
Podocalyxin
GLEPP1
Podocin
Synaptopodin
P57
VEGF
PAX2 (+/-)
Ki-67 (+/-)
Cytokeratin

WT1
Synaptopodin
CD68

Podocalyxin
GLEPP1
Podocin
Synaptopodin
P57
Cytokeratin

Capillary loop stage

Podocalyxin
GLEPP1
Cytokeratin (+/-)

WT1
Podocalyxin
GLEPP1
Podocin
Nephrin
CD2AP
Vimentin
CALLA

WT1
Synaptopodin
CD68

Podocalyxin
GLEPP1
Podocin
Synaptopodin
P57
VEGF
CALLA
Ki-67 (+/-)

PAX2 coexpression with WT1
Podocalyxin
GLEPP1
Podocin
Synaptopodin
P57
Cytokeratin

Cytokeratin
PAX2
CD68
Mature stage

- PAX2
- GLEPP1
- Cytokeratin
- Vimentin
- Ki67, PCNA (+/-)
- Podocalyxin ?

+ WT1
- Synaptopodin
- Podocalyxin
- GLEPP1
- Podocin
- Nephrin
- CD2AP
- Vimentin
- Synaptopodin
- p57
- VEGF
- CALLA
- Ki-67 (+/-)

+ PAX2 coexpression with WT1
- Podocalyxin
- GLEPP1
- Podocin
- Synaptopodin
- Nephrin
- Vimentin
- p57
- VEGF
- α-actinin-4

- Cytokeratin
- CD68
- p27, p57
- Podocalyxin ?
Markers used for podocyturia diagnostics