Editorial Review

The urinary sediment beyond light microscopical examination

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In the present issue of NDT, Chan et al. [1] report that the messenger (m)RNA expression of T-bet (a central Th1 transcription factor), interleukin (IL)-10, transforming growth factor (TGF)-β and MCP-1 in the urinary sediment of patients with active lupus nephritis decrease with successful immunosuppressive therapy. Importantly, various other cytokine mRNAs, including those of IL-2, -4, -12 and -18 did not change during the 6 month follow-up period. These observations extend to a number of prior studies by the same group [2,3] and add to a growing list of molecules (pro-inflammatory cytokines, fibrosis markers, etc.) that have been assessed in the urinary sediment of patients with various renal diseases (Table 1).

In the present review, we will summarize the key findings related to urinary mRNA measurements and critically discuss the value of these novel approaches.

Primary glomerular disease and chronic kidney disease (CKD)

Damage to, and loss of, podocytes is a key pathogenic event in proteinuria and in progressive CKD. Options to monitor this more specifically than via proteinuria include the demonstration of shedded podocytes in the urine [4]. In experimental models, podocyturia antedates proteinuria and later decreases, despite the persistence of proteinuria. An alternative to the search of podocytes by flow-cytometry is the option to assess mRNA levels of podocyte-specific proteins, such as nephrin and podocin. Urinary mRNA levels of both correlate with the rate of decline in renal function [5]. These studies suggest that the non-invasive assessment of podocyte damage and loss better reflects ‘active’ glomerular injury than proteinuria and that it can differentiate between ongoing injury and residual changes due to altered glomerular morphology.

Other studies approached CKD by focussing on central mediators of the inflammation–fibrosis sequence [6,7]. An increased mRNA and/or protein expression of TGF-β1, a central pro-fibrogenic growth factor, has been detected within cellular and fibrocellular crescents as well as in tubular epithelial cells in renal diseases [6,7]. Urinary TGF-β1 mRNA levels correlated significantly with the estimated glomerular filtration rate (GFR), the degree of tubulointerstitial scarring, and with its intra-renal expression in glomeruli and tubulointerstitium revealed by immunohistochemistry, but not with TGF-β1 urinary levels [7]. Importantly, in the same study, urinary MCP-1 mRNA expression only correlated with the degree of glomerulosclerosis and its intraglomerular presence, but not with tubulointerstitial scarring or the tubulointerstitial MCP-1 protein expression [7]. Taken together, these studies suggest that in particular, TGF-β1 mRNA in urine may serve as a non-invasive tool to assess intrarenal damage in CKD.

Lupus nephritis

In patients with systemic lupus erythematosus (SLE), the expression of TGF-β1 and MCP-1 mRNA in the urinary sediment was significantly elevated in the active disease and correlated with indices of clinical (SLEDAI) and histological disease activity. In contrast, only the urinary protein concentration of MCP-1, but not that of TGF-β1, correlated with the SLEDAI score, and neither the protein concentration in the urine correlated with the histological activity index [2]. The same group also reported that urinary IFNγ mRNA expression correlated with the overall SLEDAI score and the SLEDAI renal score. Similarly urinary IL-2 but not IL-4 mRNA expression increased in active lupus nephritis [3].

Others suggest that levels of chemokine or growth factor mRNAs in urine could distinguish class IV lupus nephritis (LN) from others, with a sensitivity of 85% and a specificity of 94%, and with an accuracy
greater than the currently available clinical markers such as SLEDAI, proteinuria, renal function or urinalysis. A significant reduction of interferon-inducible protein 10 (IP-10), -CXCR3, TGF-β and VEGF mRNA levels from baseline levels was observed in patients who responded to therapy, whereas the levels tended to increase in those who were non-responsive to treatment [8].

**Diabetic nephropathy**

Connective tissue growth factor (CTGF) is over-expressed in diabetic nephropathy (DN) and has therefore been implicated in its pathogenesis. Plasma CTGF levels and urinary CTGF protein excretion were increased in diabetic mice and strongly correlated with albuminuria [9]. Expression of CTGF mRNA in the urinary sediment has not been assessed so far in diabetic patients.

**Renal allograft**

Many authors have investigated the possibility of non-invasively diagnosing acute allograft rejection, by measuring mRNA for target genes involved in lymphocyte activation or in apoptosis or inflammation. For example, mean mRNA levels of the cytotoxic molecules perforin and granzyme B were higher in urinary cells from patients with a biopsy-confirmed episode of acute rejection compared with recipients without acute rejection [10]. Acute rejection could be predicted with a sensitivity and specificity of around 80% in both cases. In addition, in sequential urine samples, increases in perforin or granzyme B mRNA levels identified those in whom acute rejection developed. In a similar manner, granulysin mRNA in the urine increased during 11 of 14 acute rejection episodes and in follow-up studies, predicted delayed acute rejection episodes, weeks before serum creatinine started to rise [11]. Granulysin induction was associated with enhanced RANTES mRNA expression in 8 of 11 samples, whereas other molecules (granzyme B, perforin, FasL, tumour necrosis factor (TNF)-α, IL-2, IL-10, INFγ, TGF-β1, CD3 and CCR1) exhibited lower specificity and sensitivity in that study.

Another protein of interest in predicting acute rejection is CD103. It is expressed on the cell surface of alloreactive cytotoxic CD8-T lymphocytes (CTLs) and is a critical component for the intraepithelial homing of T cells. CD103 mRNA levels were higher in the urinary cells from 30 patients with an episode of acute rejection compared with those of transplant patients with other allograft pathologies [12]. Acute rejection was predicted with a sensitivity of 59% and a specificity of 75%. Urinary mRNA encoding interferon-inducible protein-10 (IP-10) and the chemokine receptor CXCR3 have also been found to predict

<table>
<thead>
<tr>
<th>Clinical scenario</th>
<th>Study</th>
<th>mRNA in urinary cells</th>
<th>Key findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary glomerular disease and chronic kidney disease</td>
<td>Szeto et al. [5]</td>
<td>Nephrin/podocin</td>
<td>Both increase in proteinuria glomerulonephritis</td>
</tr>
<tr>
<td>Lupus nephritis</td>
<td>Chan et al. [2]</td>
<td>TGF-β1, MCP-1</td>
<td>Correlation with GFR and the degree of tubulointerstitial scarring</td>
</tr>
<tr>
<td>Renal allograft</td>
<td>Li et al. [10]</td>
<td>Perforin, Granzyme B</td>
<td>High expression in allograft acute rejection</td>
</tr>
<tr>
<td>Renal allograft</td>
<td>Kotsch et al. [11]</td>
<td>Granulysin</td>
<td>High expression in allograft acute rejection</td>
</tr>
<tr>
<td>Renal allograft</td>
<td>Ding et al. [12]</td>
<td>IL-10, CXCR3, TGF-β1</td>
<td>High expression in allograft acute rejection</td>
</tr>
<tr>
<td>Renal allograft</td>
<td>Tatapudi et al. [13]</td>
<td>FOXP3, MCP-1</td>
<td>High expression in allograft acute rejection</td>
</tr>
<tr>
<td>Renal allograft</td>
<td>Muthukumar et al. [14]</td>
<td>TGF-β1</td>
<td>High expression in allograft acute rejection</td>
</tr>
<tr>
<td>Renal allograft</td>
<td>Mas et al. [15]</td>
<td>TGF-β1</td>
<td>High expression in CAN</td>
</tr>
</tbody>
</table>

Table 1. Studies investigating the cellular content of specific mRNAs in the urinary sediment
acute rejection with a sensitivity of 100% and a specificity of 78% (IP-10) and 63 and 83% (CXCR3), respectively. Immunohistological analysis of allograft biopsies showed an exuberant expression of IP-10 and CXCR3 during acute rejection, whereas both were absent in grafts with stable function [13]. Finally, Muthukumar et al. [14] suggest that urinary mRNA levels of FOXP3 could predict the reversibility of acute rejection and graft loss after an episode of acute rejection. FOXP3 is a specific marker of Treg cells (i.e. regulatory T lymphocytes), a specialized subgroup of CD4+CD25+ T lymphocytes involved in the suppression of autoimmunity and in transplantation tolerance. FOXP3 mRNA levels were higher in urine in the group with acute rejection, than in the group with chronic allograft nephropathy (CAN) or in the group with normal renal biopsy results. FOXP3 mRNA levels were inversely correlated with serum creatinine levels measured at the time of the biopsy in the acute rejection group, but not in the group with CAN or in that with normal renal biopsy results. Reversal of acute rejection could be predicted with 90% sensitivity and 73% specificity. Also, FOXP3 mRNA levels identified subjects at risk for graft renal failure within 6 months after the acute rejection episode [14]. Taken together, these various studies raise hope that urinary mRNA levels of key effectors or regulators of the immune response can be used to predict or diagnose acute allograft rejection non-invasively.

With respect to CAN, TGF-β1 mRNA levels in urinary cells were higher in CAN patients compared with recipients with stable graft function, with or without proteinuria exceeding 500 mg/24 h [15]. Epidermal growth factor (EGF) mRNA levels in urinary cells, in contrast, were reduced in CAN patients but also in those with stable graft function, but with a proteinuria of about 500 mg/24 h [15].

Chances and potential pitfalls

The above data demonstrate that mRNA levels of various genes in the urinary sediment cells may become useful in a variety of clinical situations. Compared with the measuring of protein levels, e.g. of cytokines or growth factors in the urine, mRNA determinations avoid the problems in glomerular filtration and tubular reabsorption that has plagued protein measurements. Especially in the case of cytokines and growth factors, most of which are low-molecular weight proteins, it was often observed that their urinary levels strongly correlated with albuminuria suggesting that significant amounts were derived from glomerular filtration. In this case, determination of such individual proteins would offer no more information than any assessment of proteinuria, in particular low-molecular weight proteinuria. All of this can be avoided if cells in the urinary sediment are examined and mRNA is extracted from them. This assumption is well-supported by the data mentioned previously where, in various patient groups only TGF-β1 mRNA but not TGF-β1 protein in the urine correlated with disease activity [4,6].

However, before urinary mRNA measurements (or the detection of specific cells in the urine) can be integrated into routine clinical practice, it is important to realize that technical issues related to the pre-analytic phase still need to be addressed in a more systematic manner. Thus, much remains unknown about the stability of cells and their mRNA in urine, what the optimum collection method is (i.e. spot vs timed collection), whether there is a circadian variation or whether urine should be collected in the presence of RNase inhibitors, etc. Until such information is available urinary mRNA levels will remain experimental tools.

Conflict of interest statement. None declared.

(See related article by Chan et al. NDT Advance Access publication January 31, 2006. doi:10.1093/ndt/gfk102.)

References


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