Pathological Significance of a Panel of Urinary Biomarkers in Patients with Drug-Induced Tubulointerstitial Nephritis

Yu Wu, Li Yang, Tao Su, Chen Wang, Gang Liu, and Xiao-mei Li

Renal Division, Department of Medicine, Peking University First Hospital, Beijing, China; Institute of Nephrology, Peking University, Beijing, China; and Key Laboratory of Renal Disease, Ministry of Health of China, Beijing, China

Background and objectives: Although a renal biopsy is indispensable for depicting the severity of pathologic lesions in drug-induced tubulointerstitial nephritis (DTIN), it is not acceptable in some cases and cannot be performed serially because of its invasive nature. Therefore, the discovery of noninvasive markers that are closely related to the pathology of DTIN is of great value.

Design, setting, participants, & measurements: In this study, the urinary levels of monocyte chemotactic peptide-1 (MCP-1), neutrophil gelatinase-associated lipocalin (NGAL), N-acetyl-β-d-glucosaminidase, and α1-microglobulin were measured in 40 DTIN subjects, and the performances of these parameters for distinguishing different pathologic lesions were compared.

Results: Linear correlation and receiver operating characteristic curve analyses showed that urinary MCP-1 levels were able to identify serious interstitial edema and inflammatory infiltration with greater accuracy than the other biomarkers (r = 0.501, P < 0.001 and r = 0.768, P < 0.001, respectively), whereas urinary NGAL levels showed the highest correlation coefficient with tubular atrophy (r = 0.692, P < 0.001).

Conclusions: These results suggest that these biomarker levels were higher in patients with DTIN than in controls. Urinary MCP-1 levels correlated and were predictive of the gradated severity of acute lesions in DTIN, whereas the roles of NGAL and α1-microglobulin in chronic alterations require further study.


Tubulointerstitial nephritis (TIN) is pathologically characterized by tubular injuries, interstitial inflammation, and fibrosis. A renal biopsy is needed to depict the injury severity, inflammation, and occurrence of chronic lesions. However, because a biopsy is invasive, it is not acceptable in some cases and cannot be performed serially. Thus, it is extremely valuable to develop reliable and noninvasive markers that have close correlations with the pathologic lesions to help clinicians evaluate the degree of the pathology, establish an appropriate therapy strategy, and predict the outcome.

Recently, biomarker discovery, in particular focusing on acute kidney injury (AKI), has been a major area of study, but there is limited knowledge about the utility of biomarkers in patients with TIN. Among the plethora of proteins, we chose monocyte chemotactic peptide-1 (MCP-1) and neutrophil gelatinase associated lipocalin (NGAL), given their roles in the proinflammatory response (1,2), which potentially could be able to reflect the degree of inflammation in our TIN patients. We also wanted to measure biomarkers that can reflect the severity of tubular injury, because the tubules can never be intact while the interstitial area surrounding them presents inflammation or fibrosis in TIN cases. Urinary levels of N-acetyl-β-d-glucosaminidase (NAG) have been shown to correlate well with tubular necrosis, whereas the increase in urinary α1-microglobulin (α1-MG) levels reflects the dysfunction of tubular reabsorption of small molecule proteins (3). The measurement of these two biomarkers is relatively inexpensive and simple and also has been widely applied in our clinical practice. In this study, we hypothesized that the urinary levels of MCP-1, NGAL, α1-MG, and NAG are related to the amount of tubulointerstitial damage and investigated their correlations with the degree of renal morphologic damage in patients with drug-induced tubulointerstitial nephritis (DTIN).

Materials and Methods

DTIN Patients

This study was approved by the Committee on Research Ethics of the Peking University First Hospital. A total of 40 patients (11 men and 29 women; mean age, 46 years; range, 18 to 72 years), who were clinicopathologically diagnosed with DTIN from January 2001 to May 2008, were included in this study. The diagnosis of DTIN was made when the patients met the following criteria: (1) presence of serum creatinine (Cr) above normal range (133 μmol/L) that was related to exposure to a culprit drug and (2) presence of obvious tubular dysfunction such as small-molecular-weight proteinuria, glycosuria, renal tubular acidosis, Fanconi syndrome, and urinary sediment abnormalities (sterile leukocyturia, hematuria).

The exclusion criteria were as follows: (1) TIN caused by primary glomerular diseases, autoimmune diseases, connective tissue diseases, and serious and active infections and tumors; (2) cases accompanied by infectious diseases, obstructive nephropathy, cystic nephropathy, renal artery stenosis, diabetes mellitus, active liver diseases, liver cirrhosis,
serious heart diseases, lung interstitial fibrosis, chronic obstructive pulmonary disease, or malignant tumors; and (3) either a history of >10 years of hypertension or proteinuria of >3 g/24 h.

Control Subjects
The control group in this study was specifically matched on age and gender with the TIN group. They were selected from our healthy faculty volunteers. Urine examination was done, and cases with infection and nephropathy were excluded. Twenty healthy volunteers (6 men and 14 women) were enrolled from 2001 to 2008.

Urine and Plasma Samples
Plasma and early morning midstream urine samples were collected from all patients on the morning of a renal biopsy. For female controls and patients, urine samples were obtained during their intermenstrual period to avoid the disturbance of menstrual blood on the observation of hematuria after biopsy. The obtained blood and urine samples were centrifuged at 2500 rpm for 10 minutes within 2 hours of collection. The plasma and urinary supernatants were frozen and stored at −80°C until simultaneous analysis using the same kits.

Pathologic Studies
All kidney biopsy sections were processed for light microscopy examination (hematoxylin and eosin, periodic acid-Schiff, and Masson’s trichrome staining). We routinely have two pathologists reading renal biopsy histology and making scores. If there was any discrepancy in the scoring, a nephrologist in our institute who was qualified as a renal pathologist read the slide and decided the final score. All of the pathologists were blinded to the intent of the study and to the biomarker levels. The tissue core was often obtained at depths of approximately 1 cm. The parameters for tubular atrophy and interstitial lesions (such as interstitial edema, inflammatory infiltration, and fibrosis) were assessed by two kinds of scoring systems. The area semiquantitative scores were assessed according to the proportion of the lesion area relative to the total section area and classified by a modification of the Banff 97 classification (4) as follows: score 1, <25%; score 2, 25 to 50%; score 3, 50 to 75%; score 4, >75%. The degree semiquantitative scores were classified into scores of 1, 2, and 3, corresponding to mild, moderate, and severe, respectively. The degree semiquantitative scores for interstitial infiltration were defined as follows: score 1, foci with <50 infiltrated mononuclear cells (single or multiple foci) or <50 cells per high-power field (diffuse changes); score 2, foci with 50 to 100 infiltrated mononuclear cells (single or multiple foci) or 50 to 100 cells per HPF (diffuse changes); score 3, foci with >100 infiltrated mononuclear cells (single or multiple foci) or >100 cells per HPF (diffuse changes). The degree semiquantitative scores for fibrosis were defined as follows: score 1, scattered focal fibrosis; score 2, scattered diffuse fibrosis; score 3, extensive diffuse fibrosis.

Serum and Urinary Cr and Urinary α1-MG and NAG Measurements
The serum and urinary Cr levels were measured by a picric acid colorimetric assay. The urinary α1-MG and NAG levels were measured by immune transmission turbidity and a spectrophotometric method, respectively. To compensate for differences in urine flow rate (5), urinary excretion of biomarkers was normalized for millimoles of urinary Cr. The results were expressed as microgram per millimoles Cr for α1-MG and units per nanomoles Cr for NAG. The inter- and intra-assay coefficient of variations were 5 to 10% for α1-MG and 5.3 to 7.0% for NAG.

Urineary MCP-1 and NGAL Measurements
The urinary MCP-1 and NGAL levels were measured by specific ELISA methods (Jingmei, Beijing, China, and Antibodyshop, Gentofte, Denmark, respectively) according to the manufacturers’ protocols. The lower limits of detection were 15 pg/ml for MCP-1 and 4 pg/ml for NGAL. Urinary levels below these limits were considered undetectable and expressed as zero. Urine MCP-1 and NGAL levels were standardized to urinary Cr measured in the same spot urine and expressed as nanograms per millimoles Cr and micrograms per millimoles Cr, respectively. The inter- and intra-assay coefficient of variations were 5 to 10% for MCP-1 and 1.3 to 4.0% for NGAL.

Statistical Analyses
Statistical analyses were performed using the software SPSS Version 11.0 (SPSS Inc., Chicago, IL). Differences between groups were analyzed by the nonparametric Kruskal-Wallis test. Correlations were assessed according to the Pearson test for parametric data and the Spearman test for nonparametric data. Control subjects were not included in correlation analyses. Differences in urinary biomarker levels between the patient groups and healthy controls were analyzed by the Mann-Whitney U-test. A two-sided P < 0.05 was considered statistically significant. To distinguish different pathologic lesions, receiver operating characteristic (ROC) curve analyses of the urinary biomarker levels were carried out to determine the area under the curve (AUC) and to calculate the sensitivity and specificity using the most discriminate thresholds.

Subject Characteristics
The demographic and clinical data of the subjects are summarized in Table 1. No significant differences were detected with respect to age and gender. The DTIN patients exhibited lower levels of hemoglobin and higher BP, urinary micro albumin, and serum Cr compared with the controls. The drugs responsible for the DTIN episodes were identified as antibiotics in 13 patients, nonsteroidal anti-inflammatory drugs in 13 patients, and Chinese herbs in the remaining 14 patients.

Urinary MCP-1, NGAL, α1-MG, and NAG Levels
Elevated urinary MCP-1, NGAL, α1-MG, and NAG levels were detected in the DTIN patients compared with the controls (Table 2).

Table 1. Demographics and clinical characteristics of the subjects

<table>
<thead>
<tr>
<th></th>
<th>DTIN (n = 40)</th>
<th>Control (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>43.90 ± 13.42</td>
<td>43.5 ± 10.53</td>
</tr>
<tr>
<td>Gender (f/m)</td>
<td>29/11</td>
<td>14/6</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>122.30 ± 16.23a</td>
<td>109.25 ± 9.38</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>78.24 ± 11.32a</td>
<td>62.1 ± 7.72</td>
</tr>
<tr>
<td>Hemoglobin (g/L)</td>
<td>106.46 ± 17.88b</td>
<td>119.3 ± 8.02</td>
</tr>
<tr>
<td>umAlb (mg/L)</td>
<td>94.31 ± 88.13b</td>
<td>1.27 ± 2.72</td>
</tr>
<tr>
<td>Scr (μmol/L)</td>
<td>202.56 ± 86.43b</td>
<td>81.7 ± 20.35</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SD. umAlb, urinary micro albumin; scr, serum creatinine.

aP < 0.05 versus controls.

bP < 0.01 versus controls.
Table 2. Comparison of urinary biomarkers in the subjects

<table>
<thead>
<tr>
<th></th>
<th>DTIN (n = 40)</th>
<th>Control (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCP-1 (ng/mmol creatinine)</td>
<td>363.25 ± 495.53a</td>
<td>8.83 ± 15.38</td>
</tr>
<tr>
<td>NGAL (µg/mmol creatinine)</td>
<td>233.39 ± 225.37a</td>
<td>0.60 ± 0.75</td>
</tr>
<tr>
<td>a1-MG (mg/mmol creatinine)</td>
<td>31.86 ± 22.86a</td>
<td>0.80 ± 0.53</td>
</tr>
<tr>
<td>NAG (u/mmol creatinine)</td>
<td>3.15 ± 2.10a</td>
<td>0.72 ± 0.34</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SD. *P < 0.01 versus control.

Correlations of Urinary MCP-1, NGAL, a1-MG, and NAG Levels and Tubulointerstitial Lesions

As shown in Table 3, the urinary MCP-1, NGAL, and a1-MG levels were significantly associated with the severity of interstitial edema, inflammatory infiltration, and tubular atrophy, whereas the urinary NAG levels showed positive correlations with the latter two parameters.

The linear correlations between the urinary MCP-1 levels and interstitial edema and inflammatory infiltration were the closest (r = 0.501, P < 0.001 and r = 0.768, P < 0.001, respectively), whereas urinary NGAL levels showed the highest correlation coefficient with tubular atrophy (r = 0.692, P < 0.001).

Urinary MCP-1, NGAL, a1-MG, and NAG as Biomarkers to Distinguish Different Tubulointerstitial Lesions

The scores for interstitial edema, inflammatory cell infiltration, fibrosis, and tubular atrophy overall in patients were 0.93 ± 0.79, 4.38 ± 2.08, 3.5 ± 1.25, and 3.0 ± 0.85, respectively. The ROC curves depict the true-positive fractions (sensitivity) and false-positive fractions (1 - specificity) at various cut-off points for urinary MCP-1, NGAL, a1-MG, and NAG levels for distinguishing different pathologic lesions. We used the third quartile value of each pathologic lesion parameter as the cut-off point.

For distinguishing interstitial edema and inflammatory infiltration, the ROC areas under the curve (AUCs) of the different urinary biomarkers were as follows: MCP-1 had the highest AUC (0.901), followed by NGAL (0.879), and a1-MG (0.797). The cut-off points that maximized the combined sensitivity and specificity for urinary MCP-1, NGAL, and a1-MG were 157.9 ng/mmol Cr, 349.7 µg/mmol Cr, and 32.0 mg/mmol Cr, respectively. At these thresholds, the sensitivities and specificities were 83.3 and 60.7% for MCP-1, 66.7 and 82.1% for NGAL, and 83.3 and 67.9% for a1-MG, respectively.

For distinguishing tubular atrophy, the AUCs for a1-MG and NGAL were 0.774 and 0.743 (P < 0.05), whereas the AUCs for MCP-1 and NAG had no statistical significance. The cut-off points that maximized the combined sensitivity and specificity for urinary NGAL and a1-MG were 168.9 µg/mmol Cr and 31.1 mg/mmol Cr, respectively. At these thresholds, the sensitivities and specificities were 90.9 and 65.6% for NGAL and 81.8 and 62.1% for a1-MG, respectively.

For distinguishing interstitial fibrosis, none of the AUCs of the different urinary biomarkers reached statistical significance (P > 0.05).

Discussion

In current clinical practice, a renal biopsy is the golden standard for diagnosis, prognostication, and therapeutic management of DTIN. It is already known that serum Cr is not a sensitive marker for either exacerbation or recovery of the disease. With a view to providing more noninvasive information without a renal biopsy for clinical use, we studied the expression patterns of a panel of urinary biomarkers, comprising MCP-1, NGAL, a1-MG, and NAG, in a cohort of patients with biopsy-proven DTIN.

In this study, the urinary MCP-1 levels showed the closest correlations with interstitial edema and inflammatory infiltration. Mononuclear cells, including lymphocytes and macrophages, are usually the predominant cell types in TIN and play key roles in the pathogenesis of tubulointerstitial injury (1,6). MCP-1 is one of the most specific and powerful chemoattractants and activating factors for monocytes (6). Therefore, it can be proposed that the urinary MCP-1 level reflects the degree of interstitial monocyte infiltration and the acute response of tubular epithelial cells, thereby reflecting an active ongoing inflammatory process in the diseased kidney that would probably benefit from immunosuppressive treatment.

The urinary NGAL levels in patients with DTIN in this study rose by >350-fold compared with controls. Monocytes/macrophages, neutrophils, and tubular epithelial cells were reported...

Table 3. Correlation between urinary biomarkers and tubulointerstitial lesions

<table>
<thead>
<tr>
<th>Indexes</th>
<th>Urinary MCP-1</th>
<th>Urinary NGAL</th>
<th>Urinary a1-MG</th>
<th>Urinary NAG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P</td>
<td>r</td>
<td>P</td>
</tr>
<tr>
<td>Interstitial edema</td>
<td>0.501</td>
<td>&lt;0.001</td>
<td>0.370</td>
<td>0.011</td>
</tr>
<tr>
<td>Inflammation infiltration</td>
<td>0.768</td>
<td>&lt;0.001</td>
<td>0.639</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tubular atrophy</td>
<td>0.563</td>
<td>&lt;0.001</td>
<td>0.692</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Interstitial fibrosis</td>
<td>-0.059</td>
<td>0.695</td>
<td>0.187</td>
<td>0.213</td>
</tr>
</tbody>
</table>
to be the major sources of NGAL production in inflammation injuries (2), which can explain the utility of NGAL as a sensitive and specific biomarker in various settings of AKI (7–9). Recently, several reports identified upregulated NGAL expression in chronic kidney disease, which was also correlated with adverse outcomes such as a rapid progression rate and requirement for dialysis (10,11). In view of the above observations, the role of this protein in chronic kidney disease needs to be further studied.

NAG has been used as an early marker of tubular epithelial cell dysfunction in many pathologic conditions (12,13). However, in our study, urinary NAG levels showed weak correlations with interstitial edema and inflammatory cell infiltration. It has been shown that enzymuria occurs rapidly after renal injury, and the secretion period is short, which may cause an early peak in NAG excretion in response to active nephrotoxicity insult that could be missed by the time DTIN patients are diagnosed (13,14).

As a low-molecular-weight (27 kD) glycoprotein, α1-MG has been used to screen for nephrotoxicity caused by environmental or occupational exposure to heavy metals at early stages (15,16). In this study, we found that α1-MG levels had the
ability to distinguish tubular atrophy with an AUC of 0.774, which may be explained by a loss of the reabsorption function by tubular epithelial cells after atrophy.

Recently, many efforts have been made to find the biomarkers for early detection, differentiation of etiology, or assessment of active inflammation and outcome of AKI. Several novel urinary biomarkers, such as kidney injury molecule-1 (17), IL-18 (18), and Na+/K+ exchanger isofrom 3 (19), have been characterized for the early detection of AKI caused by acute tubular necrosis under conditions such as cardiac surgery, transplantation, and hemolytic uremic syndrome. However, the information about biomarkers of AKI caused by acute interstitial nephritis is scarcely reported. We tried in this study to find biomarkers for the differential diagnosis of acute and chronic lesions of TIN, which could potentially assist in deciding which patients should be treated more aggressively. In this study, MCP-1 seems to function as a specific marker of active interstitial inflammation, although it has not previously been evaluated as a urinary biomarker for AKI. This result raises the possibility that MCP-1 may not only indicate acute interstitial inflammation injury but also may become a biomarker for differentiating acute interstitial nephritis from acute tubular necrosis. The latter possibility certainly needs to be verified in a larger trial in the future. On the other hand, under conditions of ischemia/reperfusion and nephrotoxicity, previous reports have shown that NGAL can detect acute tubule injury, which is one of the most common etiologies of AKI. Also, α1-MG and NAG have been studied as markers of necrotic/apoptotic damage or tubular dysfunction. Therefore, it is postulated that these three biomarkers are not specific for TIN, although they are partially associated with renal interstitial inflammation injury.

There are a number of limitations to this observational study. First, many nephrotoxins can cause TIN, and the drugs may have influenced their expression patterns. In addition, the biomarkers are increased in other types of kidney diseases, such as glomerular and vascular diseases, and are not specific to TIN. However, this study was designed to analyze the relationships between the markers and tubulointerstitial pathology, independently of the types of renal disorders and etiology. Second, kidney biopsies are representative of only a limited section of the renal parenchyma, and this would affect the results, although we tried to obtain kidney tissues at the same depth. Third, this cohort was intentionally chosen to eliminate common confounding variables, whereas the clinical situation is usually complex. Therefore, our results need to be confirmed in a larger randomized prospective trial, including patients with the usual confounding variables.

Because TIN is a heterogeneous and complex disease, it is hard to identify a “perfect biomarker” that can provide sufficient and accurate information about the disease. Thus, a panel of distinct biomarkers may be better suited for assessment of the disease process. In this study, we found that elevated urinary MCP-1 levels were quite sensitive for detecting acute lesions in patients with DTIN, whereas the roles of urinary NGAL and α1-MG in chronic alterations need further study. More studies are needed to investigate better combinations of multiple candidate biomarkers for monitoring the disease progression in TIN.

**Acknowledgment**

This work was supported by the National Key Technologies R&D Program (2007 BA104B10).

**Disclosures**

None.

**References**


