Serum Interleukin-18 as a Biomarker of Tubular Kidney Damage in Patients with Chronic Glomerulonephritis

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Abstract

Aim: to analyze the histological kidney features in patients with chronic glomerulonephritis (CGN) and arterial hypertension (AH) and without it, to assess the relationship between serum interleukin-18 (IL-18) and histological signs of tubulo-interstitial kidney lesion in patients with CGN and saved renal function.

Methods: eighty one patients with CGN. Blood samples for biomarkers were collected. The diagnosis of "chronic glomerulonephritis" was defined by the clinical, laboratory data and renal biopsies. Patients were divided into 2 clinical groups: patients with AH and without AH. We used data of renal biopsies to analyze the signs of kidney tubulo-interstitial tissue lesion in patients with CGN. Levels of serum IL-18 were measured by Bender Medsystems kits (Austria).

Results: according to the results of renal microscopy 88% patients had mesangial proliferative glomerulonephritis, 7% – membranous nephropathy, 5% – membranous proliferative glomerulonephritis. Patients with CGN and AH have more severe histological tubulo-interstitial lesion parametres than patients with CGN without AH. On the basis of rank correlation analysis we proved that serum IL-18 directly correlates with indicators of of tubulo-interstitial kidney tissue lesion.
in patients with CGN, strong direct relationship was found between the level of serum IL-18 and dystrophic changes in epithelial tubules (r = 0.81, p < 0.05). Diagnostics of dystrophic changes in epithelial tubules by determining of the serum IL-18 level is a highly sensitive and specific method, with the efficiency of 96.6%.

**Keywords:** IL-18; glomerulonephritis; pathology; diagnostics

**Introduction**

Renal biopsy remains one of the main diagnostic methods in modern nephrology. It allows determining the nature of pathological changes, to predict the effectiveness of therapy, risk of adverse outcome and rate of renal function loss. One of the most important disadvantages of this study is invasiveness and well-defined indications to its conduction. That is why there is an increased interest in biological markers that allow to evaluate the activity and stage of renal process, assume the character of morphological changes in the kidney and monitor the effectiveness of treatment [3, 4, 8, 9]. Interleukin-18 (IL-18) can provide the ability to define tubular kidney lesion [1, 6]. Implementation in routine clinical practice of the marker is limited because of currently insufficient evidence base.

Interleukin-18 – a pro-inflammatory cytokine - is involved in the reactions of both innate and acquired immunity. It is produced by a large number of cells, including macrophages, osteoblasts, cells of the renal and intestinal epithelium [1, 6]. In experimental studies has been proven its role in the pathogenesis of acute ischemic tubular necrosis [5]. Later increased excretion of IL-18 in urine in mice with acute ischemic tubular necrosis was detected, which was combined with an increase of cytokine expression in renal tissue [8]. This led to the emergence of clinical research aimed to clarify the possible role of IL-18 in the diagnosis of acute and chronic kidney injury [3, 4, 7, 8]. The lack of reliable published data on using IL-18 as a marker of kidney tubular damage in patients with chronic glomerulonephritis (CGN) makes this assumption very promising. The aim of the study: to analyze the histological kidney features in patients with CGN and arterial hypertension (AH) and without it, to assess the relationship between serum IL-18 and histological signs of tubulo-interstitial kidney lesion in patients with CGN and saved renal function.

**Design and Methods**

Eighty one patients with CGN were enrolled in the study. The design of the study has described in detail recently [10]. The diagnosis of "chronic glomerulonephritis" was defined by the clinical, laboratory data and renal biopsies. Patients were divided into 2 clinical groups: patients with AH and without AH. The first group included 49 patients with CGN and AH, 34 (69%) men, 15 (31%) women, who had an average age 36.3±2.3 years, disease duration
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87.1±9.8 months. Nephrotic syndrome with proteinuria (PU) above 3 g/l was observed in 8% of patients. Urinary syndrome with low PU and different severity of erythrocyturia was detected in 86% of patients, PU above 1 g/l – in 6%. The average daily rate of PU was 1.4±0.1 g/day. The second clinical group – 32 patients with CGN without AH, 20 (63%) men, 12 (37%) women, average age 38.7±7.5 years, disease duration 47.1±6.8 months. Urinary syndrome was manifested by low PU and different severity of erythrocyturia. In 66% of patients lab picture was defined as remission. Intermediate PU was 0.41±0.04 g/day. The control group consisted of 20 healthy persons, who were examined to clarify the standards of level markers.

The results of the main laboratory parameters of studied patients are presented in table 1.

Table 1 The main laboratory parameters of patients with CGN (M±m)

<table>
<thead>
<tr>
<th>The indicator</th>
<th>Patients with CGN and AH (n=49)</th>
<th>Patients with CGN without AH (n=32)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin, g/l</td>
<td>136.5±2.0</td>
<td>138.5±0.5</td>
<td>NS</td>
</tr>
<tr>
<td>Blood albumin g/l</td>
<td>42.8±0.7</td>
<td>44.1±0.2</td>
<td>NS</td>
</tr>
<tr>
<td>Blood cholesterol, mmol/l</td>
<td>5.6±0.3</td>
<td>4.9±0.4</td>
<td>NS</td>
</tr>
<tr>
<td>Blood creatinine, mmol/l</td>
<td>101.9±3.5</td>
<td>97.2±2.2</td>
<td>NS</td>
</tr>
<tr>
<td>Blood urea, mmol/l</td>
<td>5.8±0.2</td>
<td>5.9±0.3</td>
<td>NS</td>
</tr>
<tr>
<td>GFR (Cockroft-Gault), ml/min/1.73/m²</td>
<td>93.8±2.8</td>
<td>94.5±1.8</td>
<td>NS</td>
</tr>
<tr>
<td>GFR (CKD-EPI), ml/min/1.73/m²</td>
<td>88.7±2.2</td>
<td>91.3±2.3</td>
<td>NS</td>
</tr>
</tbody>
</table>

We used data of renal biopsies to analyze the signs of kidney tubulo-interstitial tissue lesion in patients with CGN.

Analysis of the tubule-interstitial tissue lesion was performed on the following signs: dystrophic and necrotic changes in epithelial tubules, thickening of the tubular basement membrane, presence of cellular infiltration, interstitial fibrosis [2].

Levels of serum Il-18 were measured by Bender Medsystems kits (Austria).

We evaluated the diagnostic accuracy of renal morphological changes on the level of the serum biomarker compared with biopsy by calculating operating
performance tests, which include: diagnostic sensitivity, diagnostic specificity and diagnostic efficiency.

All statistical analyses were performed in SPSS for Windows v. 7 17.0 (SPSS Inc., USA). The study data was statistically processed, parametric and nonparametric methods of variance were used to determine the reliability of the results. A calculated difference of $P<0.05$ was considered significant.

**Results**

According to the results of renal microscopy 88% patients had mesangial proliferative glomerulonephritis, 7% – membranous nephropathy, 5% – membranous proliferative glomerulonephritis.

Histological picture of patients with CGN and AH was characterized by the presence of dystrophic and necrotic changes in epithelial tubules of varying degree (98% and 31%), thickening of the tubular basement membrane (61%), presence of cellular infiltration (14%), interstitial fibrosis (100%). Microscopy data of patients with CGN without AH was differed from the previous group and had the features: dystrophic changes of epithelial tubules were defined in 31%, necrotic – in 9%, thickening of the tubular basement membrane – in 28%, presence of cellular infiltration – in 9%, fibrocellular crescents were absent.

Statistical analysis of morphological study by two groups showed the presence of significant differences, in patients with CGN and AH indicators of tubular changes were more pronounced than in patients without AH.

We investigated the serum levels of the marker in patients with CGN and control group. Results are presented in table 2.

<table>
<thead>
<tr>
<th>The indicator</th>
<th>Patients with CGN and AH (n=49)</th>
<th>Patients with CGN without AH (n=32)</th>
<th>Control group (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum IL-18, pg/ml</td>
<td>704.2: 648.6-1201.0</td>
<td>160.6: 76.5-613.6</td>
<td>98.1: 70.1-112.8</td>
</tr>
</tbody>
</table>

Level of serum IL-18 in patients with CGN was higher compared to the control group. Thus, in patients with AH IL-18 level was in 7.9 times higher, in patients without AH – in 2.9 times higher than in the control group ($p < 0.05$).

We suggested that serum IL-18 is a marker of tubulo-interstitial kidney tissue lesion, so we conducted rank correlation analysis in patients with CGN. Results are presented in table 3.
Table 3 Results of rank correlation analysis between serum IL-18 and indicators of tubulo-interstitial kidney tissue lesion in patients with CGN

<table>
<thead>
<tr>
<th>The indicator of tubule-interstitial kidney tissue lesion</th>
<th>Serum NGAL</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presence of cellular infiltration</td>
<td>r=-0.03</td>
<td>NS</td>
</tr>
<tr>
<td>Necrotic changes in epithelial tubules</td>
<td>r=+0.34</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Dystrophic changes in epithelial tubules</td>
<td>r=+0.81</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Thickening of the tubular basement membrane</td>
<td>r=+0.16</td>
<td>p&gt;0.05</td>
</tr>
<tr>
<td>Interstitial fibrosis</td>
<td>r=+0.61</td>
<td>p&lt;0.05</td>
</tr>
</tbody>
</table>

We found that level of serum IL-18 directly correlated with necrotic and dystrophic changes in epithelial tubules (r = 0.34, r = 0.81, p < 0.05) and interstitial fibrosis (r = 0.61, p < 0.05).

We evaluated diagnostic accuracy of dystrophic changes in epithelial tubules with serum IL-18 by calculating operating tests, such as diagnostic sensitivity, specificity and efficiency. Diagnostics of dystrophic changes in epithelial tubules was carried out by two methods in 81 patients with CGN: the first method – renal biopsy, which is considered the diagnostic standard, the second – the definition of serum IL-18. Results are presented in table 4.

Table 4 Results of dystrophic changes in epithelial tubules diagnostics according to renal biopsy and serum IL-18 definition

<table>
<thead>
<tr>
<th>The diagnostic method</th>
<th>Renal biopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum IL-18</td>
<td>Diagnostics</td>
</tr>
<tr>
<td></td>
<td>Dystrophic changes in epithelial tubules were present</td>
</tr>
<tr>
<td></td>
<td>Dystrophic changes in epithelial tubules were absent</td>
</tr>
</tbody>
</table>

According to the table 4 we defined diagnostic sensitivity for dystrophic changes in epithelial tubules determining by the serum IL-18, which was 93.1%, the diagnostic specificity – 100% diagnostic efficiency – 96.6%. Analysis of the operational characteristics of the dystrophic changes in epithelial tubules diagnostics by using serum IL-18 leads to the conclusion that this method is highly sensitive and specific, with the efficiency of 96.6%.

Nonlinear model depending on the presence of dystrophic changes in epithelial tubules diagnosed by serum IL-18 in patients with CGN is:
\[ D_t = \frac{1}{1 + 10000 \cdot 1.02^{-n_t}}, \]  

where \( D_t \) - the presence of dystrophic changes in epithelial tubules; \( IL_t \) - the serum level of IL-18 in the patient.

It is a high quality model as the correlation coefficient between the input range and model value is 0.91. An analysis of the constructed nonlinear model allows us to conclude that the serum level of IL-18 above 600 pg/ml indicates dystrophic changes in epithelial tubules.

**Conclusions**

1. Patients with CGN and AH have more severe histological tubular lesion parameters than patients with CGN without AH.
2. On the basis of rank correlation analysis we proved that serum IL-18 directly correlates with indicators of tubulo-interstitial kidney tissue lesion in patients with CGN, strong direct relationship was found between the level of serum IL-18 and dystrophic changes in epithelial tubules (\( r = 0.81, p < 0.05 \)).
3. Diagnostics of dystrophic changes in epithelial tubules by determining of the serum IL-18 level is a highly sensitive and specific method, with the efficiency of 96.6%.
4. The serum level of IL-18 above 600 pg/ml indicates dystrophic changes in epithelial tubules.

**References**

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http://dx.doi.org/10.1053/j.ackd.2010.09.002

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