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### Urinary excretion of soluble tumour necrosis factor receptor 1 as a marker of increased risk of progressive kidney function deterioration in patients with primary chronic glomerulonephritis

Ilona Idasiak-Piechocka¹, Andrzej Oko¹, Elzbieta Pawliczak¹, Elzbieta Kaczmarek² and Stanisław Czekalski¹

¹Department of Nephrology, Transplantology and Internal Medicine, Poznań University of Medical Sciences and ²Laboratory of Morphometry at Med Image Processing, at Department of Pathology, Poznań University of Medical Sciences, Poznań, Poland

Correspondence and offprint requests to: Ilona Idasiak-Piechocka; E-mail: ilonaidasiak@poczta.onet.pl

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**Abstract**

**Background.** The effects of tumor necrosis factor α (TNF α), a potent proinflammatory cytokine, in the kidneys are mediated by two membrane receptors (TNFR), TNFR1 and TNFR2. The expression of both TNF and TNFRs increases in several kidney diseases and is associated with the shedding of the receptors out of the cell membranes. In an experimental model of glomerulonephritis (GN), elevated concentrations of TNFRs in serum and TNFRs excretion in urine were demonstrated. The aim of this study was evaluation of urinary excretion of TNFR1 and its relationship with the clinical markers of kidney injury in patients with GN. The value of basal urinary TNFR1 excretion as a prognostic indicator of the progression of kidney function impairment was also assessed.

**Material and Methods.** Fifty-five patients with newly diagnosed, biopsy-proven primary GN were included in the study. In all patients, and in 20 healthy subjects, UTNFRI was measured using an ELISA. In the patients, risk factors of the progression of impairment of kidney function (reduced eCcr, nephrotic syndrome, hypertension and intensity of morphological lesions in the kidneys) were evaluated. The appropriate treatment was then introduced and the patients were in follow-up for 4 years. The progression of kidney function impairment was defined as a reduction of eCcr > 5 mL/min/1.73 m²/year during follow-up. The association of basal TNFRI excretion with the progression was evaluated.

**Results.** Urinary excretion of TNFR1 in the patients with GN (4039.2 ± 3801.5 pg/mgCr) was greater than in the healthy subjects (1358.9 ± 927.8 pg/mgCr, P < 0,00002). A significant negative correlation between TNFR1 excretion and eCcr (Sr=0.464, P < 0.01) and a positive correlation between TNFR1 excretion and proteinuria (Sr = 0,463, P < 0.01) were found. In 13 patients, a marked reduction of eCcr was observed during follow-up. Logistic regression analysis revealed that TNFR1 excretion > 3863.3 pg/mgCr predicts progression of renal function impairment along with advanced interstitial fibrosis in the kidney biopsy specimens at presentation.

**Conclusion.** Markedly elevated urinary TNFR1 excretion may be considered as a good marker of an activated TNFα-pathway in patients with newly diagnosed GN and as a potentially modifiable risk factor of progressive kidney function impairment.

**Keywords:** cytokines; glomerulonephritis; progression of renal diseases; TNF receptors; TNFalfa

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**Introduction**

Tumour necrosis factor α (TNFα) is a pleiotropic cytokine with proinflammatory and immunoregulatory properties. It is mainly produced by activated macrophages. In normal kidneys, TNFα is usually not detected. However, the expression of TNFα was demonstrated in endothelial, mesangial and epithelial glomerular cells and also in tubular cells after stimulation and during inflammation as was documented by *in vitro* and *in vivo* studies [1,2]. Significantly increased tissue TNFα expressions was found in renal biopsy specimens, and high concentrations of this cytokine were demonstrated in the sera and in the urine of patients with different types of glomerulonephritis (GN) [3,4]. In nephrotic nephritis rat models, the administration of exogenous TNFα results in exacerbation of glomerular injury [5]. TNFα stimulates the release of several inflammatory mediators including interleukin-1-β (IL-1-β), monocyte chemoattractant protein (MCP-1) and tumour growth factor β (TGFβ), the main regulatory factors of collagen synthesis [6,7]. It was recently suggested that TNFα released from the mesangial cells after IgA deposition activates tubular epithelial cells and induces inflammatory changes in the renal interstitium [8]. The severity of tubulointerstitial damage is one of the main factors which correlates with the deterioration of renal function in patients with chronic kidney diseases (CKD) [9].

The effect of TNFα is mediated by two high-affinity membrane receptors (TNFR), 55-kD TNFR1 and 75-kD TNFR2, located on numerous cell types. In the normal human kidney, TNFR1 is expressed in the glomeruli, and TNFR2 is usually not expressed. The expression of TNFR1 is increased in inflammatory proliferative kidney diseases such as human lupus nephritis class III/IV, according to the WHO classification [10]. In an experimental model of the immune-complex-mediated glomerulonephritis, an increased expression of TNFR2 in the glomeruli and in the interstitium was demonstrated. The role of each TNFR in immune-complex-mediated glomerulonephritis was recently proposed [11]. It was suggested that TNFR1 is involved mainly in modulation of the immune response and in the apoptotic processes, while TNFR2 plays a critical selective proinflammatory role in mediating renal injury [12,13]. Both TNFRs can be cleaved from the cell membrane to soluble forms. Soluble TNFRs may play a critical role in regulating the inflammatory response by binding and eventually neutralizing free TNFα. It was also suggested that soluble TNFRs bound with TNFα may serve as a slow-release reservoir of TNFα in the low-grade inflammatory state [14,15]. Elevated concentration of TNFR1 in the serum, interpreted as the marker of TNFα-pathway activation and strongly associated with decreased renal function in nonproteincic type 1 diabetes patients, was recently reported [16]. In patients with lupus nephritis and in the murine model of this disease, elevated levels of TNFR1 in serum and in urine have been found [17].

In the present study, the urinary excretion of TNFR1 (UTNFR1) was evaluated to identify its potential relationship with clinical markers of kidney injury in the patients with newly diagnosed primary GN. The value of baseline urinary TNFR1 excretion as prognostic indicator of the progression of kidney function impairment was also assessed.

**Materials and methods**

Fifty-five Caucasian patients (38 men and 17 women, with a mean age of 36 ± 12 years) with newly diagnosed, biopsy-proven primary chronic GN were included in the study. Patients with any concomitant disease and the symptoms or sign of acute and chronic inflammation other than glomerulonephritis were excluded from the study. All specimens of kidney tissue were obtained by percutaneous kidney biopsy, and standard examination of the cortical tissue by light microscopy and immunofluorescence was performed by an experienced morphologist.

IgA nephropathy (IgAN) was diagnosed in 19 patients, mesangial proliferative glomerulonephritis (non-IgA nephropathy, MesPGN) in 25 patients and focal segmental glomerulosclerosis (FSGS) in 11 patients. The percentage of sclerotic glomeruli (SG) was counted, and two subgroups were discerned: without and with ≥50% of SG, n = 42 and n = 13, respectively. The severity of interstitial fibrosis (IF) was estimated by a semi-quantitative scoring system and classified as mild (~ or +; n = 35) or advanced (++ or +++; n = 20) IF.

Urinary protein excretion (UPE) on the basis of 24-h urine collection was measured, and microscopic analysis of urinary sediment was performed. Nephrotic syndrome was present in 22 patients, and in the remaining non-nephrotic range, proteinuria and/or erythrocyturia was observed. Serum and urine creatinine concentrations were measured using standard laboratory procedure, and estimated creatinine clearance (eCr) using the Cockcroft and Gault formula was calculated and corrected for a standard body surface area of 1.73 m². The mean (±SD) of eCr was 74.8 ± 38.5 mL/min/1.73 m². The eCr values in mL/min/1.73 m² were considered as approximate glomerular filtration rate (GFR) values. Normal renal function was defined as GFR ≥ 90 mL/min/1.73 m².

Sixteen patients were in stage 1 of CKD; 21 patients presented stage 2 and 18 stage 3 of CKD. Arterial hypertension (blood pressure ≥140/90 mmHg or the use of anti-hypertensive medications) was present in 34 patients. The characteristics of the study group are presented in Table 1.

In all patients, fresh urine samples were collected in the morning prior to renal biopsy and before the administration of therapy. The sample was centrifuged at 1000 rpm for 10 minutes and aliquots of 1 mL were stored at −70°C until tested. Enzyme-linked immunosorbent assay (ELISAs R&D System) was used to measure urinary sTNFR1 concentration (UTNFR1). The assay measures both free sTNFR and sTNFR1 bound with TNF (Quantikine Human sTNFR1 immunoassay; R&D systems; Cat. No DRT100). The TNFR1 excretion was expressed as picograms per milligram creatinine. TNFR1 excretion evaluated in 20 healthy subjects, matched for sex and age with patients, served as control values.

**Table 1. Characteristics of the study group.** The values are given as number of patients (n) or as mean ± SD.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (n; men/women)</td>
<td>38/17</td>
</tr>
<tr>
<td>Age (years)</td>
<td>36 ± 12</td>
</tr>
<tr>
<td>Morphological form of glomerulonephritis:</td>
<td></td>
</tr>
<tr>
<td>IgA nephropathy (n)</td>
<td>19</td>
</tr>
<tr>
<td>Mesangial proliferative glomerulonephritis (n)</td>
<td>25</td>
</tr>
<tr>
<td>Focal/segmental glomerulosclerosis (FSGS) (n)</td>
<td>11</td>
</tr>
<tr>
<td>Urinary protein excretion (g/24 h):</td>
<td></td>
</tr>
<tr>
<td>In patients with nephrotic syndrome (n = 22)</td>
<td>9.3 ± 5.2</td>
</tr>
<tr>
<td>In patients with non-neprotic proteinuria (n = 33)</td>
<td>1.6 ± 1.5</td>
</tr>
<tr>
<td>eCr (mL/min/1.73 m²):</td>
<td></td>
</tr>
<tr>
<td>In patients with eCr &lt; 90 (n = 16)</td>
<td>109.6 ± 21.1</td>
</tr>
<tr>
<td>In patients with eCr &lt; 90 (n = 39)</td>
<td>60.5 ± 19.5</td>
</tr>
<tr>
<td>Blood pressure (BP) values:</td>
<td></td>
</tr>
<tr>
<td>In patients with arterial hypertension (n = 34)</td>
<td>148.4 ± 14.7/90.7 ± 9.9 systolic/diastolic BP</td>
</tr>
<tr>
<td>In normotensive patients (n = 21)</td>
<td>118.3 ± 13.4/72.6 ± 8.0 systolic/diastolic BP</td>
</tr>
</tbody>
</table>
In all patients, treatment with an ACE inhibitor and/or AT1R antagonist and dietary intervention were introduced. Twenty-five patients received statins. Other anti-hypertensive drugs (diuretics, calcium channel blockers) were also used to reach the recommended blood pressure target (<130/80 mmHg or <125/75 mmHg, when proteinuria >1.0 g/24 h was detected). In 35 patients, treatment with corticosteroids was introduced combined with cyclophosphamide in 11 patients, azathioprine and chlorambucil in 12 patients or cyclosporine in 12 patients. Patients were controlled in the nephrological out-patient department at 6-month intervals during the 4-year follow-up. At the end of follow-up, the values of eCcr were analysed, and the patients were classified as progressors when the eCcr value decreased (below normal range) ≥ 5 mL/min/1.73 m²/year during the follow-up period. The patients demonstrating stabilization or a slower decrease of eCcr were considered as non-progressors.

The initial values of UTNFR1 were compared between progressors and non-progressors. The additional risk factors of progression were age, gender and initial values of eCcr, UPE, systolic blood pressure (SBP) and the intensity of glomerular sclerosis and interstitial fibrosis were compared between the progressor and non-progressor groups.

Statistical analysis
The data are given as the mean ± standard deviation (SD) and 95% confidence intervals (CI). The correlations between two continuous variables were calculated using Spearman's bivariate correlations. The associations between categorical variables and continuous non-normally distributed variables were calculated using the non-parametric Mann–Whitney test. The differences between categorical variables were tested by a χ²-test.

Fig. 1. Mean ± 95% confidence interval urinary TNFR1 excretion (UTNFR1) in various morphological types of chronic GM (IgAN, MesPGN, FSGS).

Fig. 2. The correlation (SR, Spearman ratio) between UTNFR1 and eGFR in patients with primary chronic GN.
or Fisher's exact test when appropriate. Multiple linear regression analysis for evaluation of the association of the risk factors of the disease progression (age of the patients, SBP, UPE, UTNFR1) and eCcr values at the presentation in all patients and the association of these risk factors with the decrease in eCcr during follow-up in the progressors was performed. Forward stepwise (likelihood ratio) logistic regression analysis was used to determine the independent risk factors of faster eCcr decline, identified at initial examination of the patients. The following variables were included into the analysis: age (>60 years), male gender, the presence of eCcr <90 mL/min/1.73 m², nephrotic syndrome, hypertension, >30% of sclerotic glomeruli and advanced IF in kidney biopsy specimen and TNFR1 excretion higher than the upper limit of 95% confidence interval for mean values in non-progressors. A P-value < 0.05 was regarded as statistically significant. Statistical analysis was performed using SPSS v.17.0 and Statistica v. 8.0 (Stat Soft Inc.).

Results

In the whole group of patients with glomerulonephritis, the mean UTNFR1 was significantly higher than in healthy subjects (4039.2 ± 3801.5 vs 1358.9 ± 927.8 pg/mg Cr, respectively, P < 0.00002). Mean values of UTNFR1 were markedly higher in the patients with all three morphological types of GN than in healthy controls but did not differ significantly from each other, although the values in patients with FSGS were the highest (Figure 1).

In the patients with GFR <90 mL/min/1.73 m² at baseline, TNFR1 excretion (4893.9 ± 4192.9 pg/mg Cr) was significantly higher than in the patients with eGFR ≥ 90 mL/min/1.73 m² (2257.0 ± 1162.8 pg/mg Cr; P < 0.001). A significant negative correlation between UTNFR1 and eCcr was found (Figure 2).

In 22 patients with nephrotic syndrome, UTNFR1 (6014.7 ± 4674.2 pg/mg Cr) was significantly elevated when compared to patients with non-nephrotic proteinuria (2868.2 ± 2137.8 pg/mg Cr; P < 0.001). A significant positive correlation between UTNFR1 and UPE in patients with GN was detected Figure 3.

Mean UTNFR1 values in patients with arterial hypertension (4223.0 ± 3668.4 pg/mg Cr) were not significantly different from those in normotensive patients (3979.9 ± 3818.3 pg/mg Cr). Both systolic and diastolic BP did not correlate with the UTNFR1 values.

There was also no difference between the mean UTNFR1 in patients with >30% of sclerotic glomeruli and those with less pronounced glomerulosclerosis (4046.4 ± 2844.4 and 4152.3 ± 3946.4 pg/mg Cr, respectively), as well as in patients with mild and advanced interstitial fibrosis (4229.6 ± 4076.8 and 3948.1 ± 2988.1 pg/mg Cr). Both systolic and diastolic BP did not correlate with the UTNFR1 values.

At the end of follow-up, 13 patients were classified as progressors (mean decline of eCcr = 33.2 ± 19.1 mL/min/1.73 m²). The comparison of the incidence or initial values of the parameters considered as risk factors for faster renal function decline in the groups of patients qualified after follow-up as progressors (PG) and non-progressors (NPG). The values are given as number of patients (n) or as the mean ± SD

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PG (n = 13)</th>
<th>NPG (n = 42)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (n; men/women)</td>
<td>13/1</td>
<td>26/15</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>eCcr (mL/min/1.73 m²)</td>
<td>54.2 ± 28.5</td>
<td>81.7 ± 27.4</td>
<td>0.004</td>
</tr>
<tr>
<td>UPE (g/24 h)</td>
<td>6.2 ± 5.6</td>
<td>4.4 ± 4.9</td>
<td>n.s.</td>
</tr>
<tr>
<td>Nephrotic syndrome (n)</td>
<td>7</td>
<td>15</td>
<td>n.s.</td>
</tr>
<tr>
<td>Arterial hypertension (n)</td>
<td>10</td>
<td>24</td>
<td>n.s.</td>
</tr>
<tr>
<td>&gt;30% sclerotic glomeruli (n)</td>
<td>5</td>
<td>8</td>
<td>n.s.</td>
</tr>
<tr>
<td>advanced IF (n)</td>
<td>8</td>
<td>12</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>UTNFR1 pg/mg Cr</td>
<td>7287.5 ± 5403.3</td>
<td>3149.1 ± 2291.9</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

Fig. 3. The correlation (SR, Spearman ratio) between UTNFR1 and UPE in patients with primary chronic GN.
gender, lower eCcr values and higher UTNFR1 were found when compared to the non-progressor group. The differences between the means (±0.95 CI) of UTNFR1 in the progressors, non-progressors and healthy subjects are presented in Figure 3. The upper value of 95% CI in non-progressors (3863.3 pg/mg Cr) was admitted as the limit above which the UTNFR1 values may influence progression (Figure 4).

Neither the incidence of arterial hypertension or blood pressure values nor the incidence of nephrotic syndrome and values of UPE were different in the progressors when compared with the non-progressors.

In the whole group of patients, the multiple regression analysis revealed that the age of the patients and UTNFR1 were independently and significantly associated with the initial eCcr values, while UPE remained in the regression model.
TNF receptor 1 as a marker of progressive kidney function deterioration

**Table 5.** Odds ratios and P-values for risk factors of progression of chronic GN identified at presentation using forward stepwise (likelihood ratio) logistic regression model

<table>
<thead>
<tr>
<th>Variables</th>
<th>Odds ratio</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male Gender</td>
<td>4.29</td>
<td>&lt;0.038</td>
</tr>
<tr>
<td>Age &gt;60 years</td>
<td>0.98</td>
<td>&lt;0.322</td>
</tr>
<tr>
<td>Nephrotic syndrome</td>
<td>1.36</td>
<td>&lt;0.244</td>
</tr>
<tr>
<td>Impaired renal function</td>
<td>3.78</td>
<td>&lt;0.052</td>
</tr>
<tr>
<td>Arterial hypertension</td>
<td>1.64</td>
<td>&lt;0.200</td>
</tr>
<tr>
<td>Advanced glomerular sclerosis</td>
<td>2.07</td>
<td>&lt;0.150</td>
</tr>
<tr>
<td>Advanced interstitial fibrosis</td>
<td>4.66</td>
<td>&lt;0.031</td>
</tr>
<tr>
<td>h UTNFR1</td>
<td>4.61</td>
<td>&lt;0.032</td>
</tr>
</tbody>
</table>

Included variables were male gender, age >60 years, presence of hypertension, nephrotic syndrome, impaired renal function (eGFR <90 mL/min/1.73 m²), glomerular sclerosis (>30%), advanced interstitial fibrosis and higher than upper limit of 95% confidence interval in non-progressors urinary TNFR1 excretion (hUTNFR1).

model but with a P-value of 0.056, at the borderline of statistical significance (Table 3).

The multiple regression analysis of the data of the progres sor group demonstrated that only initial UTNFR1 re mained as independent variable associated with the decline of eCcr during follow-up in the model of regression but with probability which did not reach statistical significance (P = 0.066) (Table 4). Logistic regression analysis revealed that UTNFR1 values above the upper value of the 95% confidence interval of mean in the non-progressor group at presentation may be considered as a significant predictive factor for the progressive impairment of renal function along with male gender and advanced interstitial fibrosis in the kidney biopsy specimens (Table 5).

**Discussion**

The results of our study indicate that in patients with newly diagnosed primary GN, the excretion of TNFR1 (UTNFR1) is significantly higher than in healthy persons. Elevated UTNFR1 levels were detected in patients with IgAN, MesPGN and FSGS and did not differ significantly from each other, although the highest values were found in patients with FSGS. This increased UTNFR1 may be attributed to the phenomenon of shedding these receptors from the membranes of glomerular cells activated by TNFα in response to the immunological injury. It was demonstrated that most renal cells produce and excrete TNFα after stimulation. In different forms of GN, like IgA nephropathy or proliferative lupus nephropathy, the expression of TNFα is upregulated preferentially in glomeruli and correlates with the increased levels of this cytokine in serum and urine [4,18–20]. These observations indicate that, in patients with primary GN free of other inflammatory processes and con comitant diseases, injured kidneys are the source of increased TNFα production and excretion. Lai et al. [24] demonstrated recently that in patients with IgAN the IgA deposition in glomerular mesangium induces TNFα synthesis by the mesangial cells and the podocytes. The authors provided the data supporting the hypothesis that TNFα derived from podocytes further upregulates the TNFα production in an autocrine manner [24]. TNFα derived from mesangial cells and podocytes upregulates the expression of TNF receptors [24]. This mechanism leading to increased TNFR1 shedding from the membranes of mesangial cells and podocytes may explain increased UTNFR1 demonstrated in our patients with IgAN, and most probably the same mechanism may operate also in patients with MesPGN. According to the hypothesis of Lai et al. [24], the binding of TNFα to TNFR1 on the glomerular cell membranes leads to interleukin 6 (IL-6) synthesis and apoptosis, while binding to TNFR2 maintains proinflammatory cellular responses. Several mediators, including mainly TNFα, released from injured glomeruli activate the tubular epithelial cells and lead to additional production of the cytokines, including TNFα and subsequent inflammatory changes in the renal interstitium [24]. It may be suggested that in patients with FSGS participating in our study, the higher, although not significantly, UTNFR1 values when compared to patients with IgAN and MesPGN are due to enhanced damage of podocytes, which results in increased TNFα production and TNFR1 expression.

The pathways involved in the proinflammatory role of TNFα in inflammatory kidney disease was recently presented by Ernandez and Mayadas [14]. These authors provided evidence that both forms of TNFα, the membrane-bound form and the soluble form, bind equally well with TNFR1, whereas TNFR2 has a higher affinity for the membrane-bound form and a low-binding capacity for the soluble form of TNFα. The increased expression of TNFR1 in the glomeruli was described in inflammatory proliferative kidney diseases, such as human lupus nephritis (WHO class III/IV). Both TNFRs can be cleaved from the cellular membranes to functional soluble forms. In patients with systemic lupus erythematosus, high serum levels of TNFα were accompanied by elevated concentrations of soluble TNFRs and correlated with the disease activity [21,22]. Elevated levels of TNFα and soluble TNFRs in serum were also reported in nonproteinuric patients with type 1 diabetes [16]. These data support the hypothesis that, in patients with GN, increased urinary TNFR1 excretion reflects increased TNFR shedding from the cell membranes and may be interpreted as a marker of increased TNFα expression in activated renal cells.

The significant negative correlation between urinary TNFR1 excretion and eCcr demonstrated in patients with GN at presentation suggests that elevated TNFR1 excretion represents activation of the TNFα pathway in the kidney, which contributes to renal function decline. The result of multiple regression analysis in the whole group of patients with GN which confirmed significant association of UTNFR1 with eCcr values at presentation supports this suggestion. This suggestion corroborates the hypothesis presented recently by Niewczas et al. [16] in the study performed in patients with type 1 diabetes. The authors demonstrated that in nonproteinuric patients with type 1 diabetes, serum concentrations of sTNFR1, sTNFR2 and soluble Fas contributed independently to GFR values measured by serum cystatin C concentration [16]. The serum concentrations of sTNFR1 and sTNFR2 were highly correlated and showed nearly the same association with GFR,
but the effect of sTNFR1 concentration on GFR was the most pronounced and was hardly changed by multivariate adjustment. The association of sTNFRs serum concentration with GFR was independent of the association of these markers with the albumin excretion rate and was interpreted as a possible indicator of the involvement of elevated serum concentrations of sTNFRs, by themselves or as a marker of activation of a TNFα pathway in early renal function decline in patients with type 1 diabetes [16]. The results of our study indicate that urinary excretion of TNFR1 also represents a marker of activation of TNFα pathway in the kidneys, which contributes to renal function decline. In the study by Niewczas et al. [16], a significant but poor correlation between TNFα and sTNFR1 concentration in serum was demonstrated. However, the assay for TNFα used by these authors only measured a free form of TNFα. If so, circulating TNFα bound to its soluble receptors could not be detected, explaining the poor correlation between the serum levels of TNFα and TNFRs. The method of determining soluble TNFR1 (sTNFR1) used in our study detects both free sTNFR1 and sTNFR1 bound to TNFα [Quantikine Human sTNF R1 Immunoassay; cat. nr DRT 100]. It seems reasonable to admit that the elevated excretion of TNFR1 demonstrated with this method is a better indicator of increased TNFα production in the kidney than free TNFR1 levels in serum.

The study of Wu et al. [17] provided evidence that inflamed kidneys represent an important source of TNFR1 in the urine of patients with lupus. In patients with newly diagnosed primary GN devoid of any other sign of inflammation, the kidney is most probably the unique source of TNFR1 excreted in urine. In the patients with GN participating in our study, TNFR1 excretion correlated positively with initial UPE. Greater UPE is considered as the indicator of more advanced glomerular barrier function impairment and as an important factor promoting tubulointerstitial tissue damage, which is caused by the infiltration of this tissue by mononuclear cells and the acceleration of fibrotic processes leading to a faster decline of the kidney function [9,23,24]. The macrophages and lymphocytes infiltrating the interstitium may be an additional source of TNFR1 excreted in urine. It was demonstrated that mononuclear cells infiltrating interstitial tissue in acute allograft rejection highly express TNFR1 [18]. Increased expression of TNFR1 in the interstitium may explain why in patients with nephrotic syndrome urinary excretion of TNFR1 was significantly greater than in non-nephrotic patients. The presence of arterial hypertension did not influence TNFR1 excretion in our patients with glomerulonephritis.

The therapy applied in the patients with GN followed up for 4 years appeared ineffective in protection against eCr reduction below 5 mL/min/1.73 m²/year in 13 patients (progressors). Four factors evaluated in the patients at presentation were significantly different in the progressors in comparison to the non-progressors: higher male/female ratio, lower eCr values, advanced interstitial fibrosis in kidney biopsy specimens and higher TNFR1 excretion. Lower GFR values at presentation, male gender and advanced histological lesions of the kidneys are established risk factors of the progressive fall of GFR in patients with CKD [25,26]. Markedly elevated urinary TNFR1 excretion detected at presentation in patients with GN may be considered as an additional risk factor for progressive GFR deterioration. The result of multiple regression analysis confirms the suggestion that UTNFR1 measured in GN patients at presentation is a better predictor of poor outcome than other classical factors of disease progression, including initial urinary protein excretion. High TNFR1 excretion can be modified by a specific treatment inhibiting TNFα expression in the kidneys. In different experimental models of glomerulonephritis in rats, the blockade of TNF reduced renal injury [27]. The reduction of glomerular injury with the reduction of glomerular interleukin-1β (IL-1β) expression was demonstrated after administration of the soluble fragment of extracellular domain of TNFR1 in the short-term model of LPS-enhanced nephrotoxic nephritis [28]. In another model of crescentic glomerulonephritis, the reduction of tubulointerstitial scarring and preservation of renal function was achieved after the treatment with a monoclonal antibody to TNFα, started at the stage at which the disease was established [29]. The results of experimental studies indicate that TNF blockade can inhibit the expression of other proinflammatory cytokines in the glomeruli and that TNFα is important both for acute inflammatory response within the kidney and for subsequent renal fibrosis [28]. The results of our study do not permit conclusions to be drawn as to conclude whether TNFR1 excretion is related to interstitial fibrosis or glomerulosclerosis. There are no controlled studies evaluating the efficacy of a TNF blockade in humans with primary chronic GN. In patients with Wegener granulomatosis (only approximately 50% of cases had evidence of renal involvement), the use of a TNF blockade with a soluble TNF receptor fusion protein (etanercept) was not found to be a significant benefit in comparison with conventional therapy [30]. Further studies are needed for evaluation of the use of TNFα blockade in treatment of patients with ANCA-associated glomerulonephritis and in other forms of glomerulonephritis.

In the logistic regression model analysis of our results, in which seven known risk factors for progressive kidney function impairment and TNFR1 excretion exceeding 3863.3 pg/mg Cr detected in the initial evaluation of the patients (upper limit of 95% CI of the mean TNFR1 excretion in the non-progressor group) were included, only three factors: male gender, advanced interstitial fibrosis and elevated UTNFR1 values were identified as important risk factors for the disease progression, in spite of the conventional treatment for 4 years. It is not known why markedly elevated urinary TNFR1 excretion is associated with progressive kidney function impairment in patients with newly diagnosed primary GN. Our findings may support the hypothesis that elevated urinary TNFR1 excretion is a good marker of increased activation of the TNFα pathway within a kidney injured by immunological processes in patients with IgAN and MesPGN or by the factors causing podocyte damage in the FSGS. The consequences of this increased activation of the TNFα pathway would be the same as suggested in the study performed in patients with type 1 diabetes in which only the serum concentration of TNFR1, TNFR2 and sFas were associated with GFR in multivariate analysis [16]. It was demonstrated that the exposure of human kidney cells to sTNFRs results in an apoptotic re-
TNF receptor 1 as a marker of progressive kidney function deterioration response and that this effect was more pronounced after exposure to sTNFR1 than to sTNFR2 [31–33]. In addition, the TNFα pathway activation directly increases glomerular vasoconstriction and albumin permeability, and the exposure of the kidney to TNFα increases mRNA expression of TNF receptors in renal tubulointerstitial and triggers cell death [16]. All these consequences of the activation of the TNF pathway induced by the injury of glomerular cells in glomerulonephritis may be involved in progressive kidney function impairment. In addition, the inflammation and fibrosis of tubulointerstitial tissue may accelerate this process.

A limitation of this study is the lack of the measurements of serum levels of TNFR1, which did not permit the correlation of UTNFR1 with circulating levels of the receptor. It was, however, already demonstrated in the experimental study that the induction of immunological kidney injury results both in the increased urinary TNFR1 excretion and increased serum TNFR1 level [34]. Therefore, the detection of increased serum TNFR1 level in patients with GN and high UTNFR1 does not permit to differentiate between its renal or extrarenal origin. To limit the potential influence of the extrarenal sources on TNFR1 urinary excretion, only patients without any concomitant disease and inflammatory process other than GN were included in the study.

We did not evaluate the expression of TNFR1 in the kidney biopsy specimens, and therefore, we were not able to identify directly particular kidney cells with increased expression of TNFR1. Furthermore, the group of newly diagnosed patients with glomerulonephritis was small and heterogeneous, but increased UTNFR1 in all three forms of GN in comparison with healthy controls was detected, which indicates indirectly the activation of the TNFα pathway as a common feature in GN. The relevance of TNFR1 in GN warrants investigation.

In conclusion, this study provides evidence that markedly elevated urinary TNFR1 excretion may be considered as a good marker of an activated TNFα pathway in patients with newly diagnosed primary GN and as a risk factor of progressive kidney function impairment which is potentially modifiable by the appropriate treatment. Further studies are needed for the evaluation of the efficacy and safety of treatment with TNFα inhibitors or TNFR blockers in patients with glomerulonephritis and a markedly elevated urinary TNFR1 excretion.

Conflict of interest statement. None declared.

References
Treatment with microemulsified cyclosporine in children with frequently relapsing nephrotic syndrome

Kenji Ishikura¹, ⁷, Norishige Yoshikawa², Shinzaburo Haţtori³, Satoshi Sasaki⁴, Kazumoto Iijima⁵, Koichi Nakanishi⁶, Takeshi Matsuyama⁷, Nahoko Yata⁴, Takashi Ando⁸, Masataka Hondaⁱ and for Japanese Study Group of Renal Disease in Children

¹Department of Pediatric Nephrology, Tokyo Metropolitan Children’s Medical Center, Fuchu, Japan, ²Department of Pediatrics, Wakayama Medical University, Wakayama, Japan, ³Department of Fundamental Medicine, Kumamoto Health Science University, Kumamoto, Japan, ⁴Department of Pediatrics, Hokkaido University Graduate School of Medicine, Sapporo, Japan, ⁵Department of Pediatrics, Kobe University Graduate School of Medicine, Kobe, Japan, ⁶Department of Pediatrics, Fussa Hospital, Fussa, Japan, ⁷Department of Clinical Research, Tokyo Metropolitan Children’s Medical Center, Fuchu, Japan and ⁸Department of Economic History, School of Economics and Management, Lund University, Lund, Sweden

Correspondence and offprint requests to: Kenji Ishikura; E-mail: kenzo@ii.e-mansion.com

Abstract

Background. We previously established a treatment protocol for conventional cyclosporine (Sandimmune, Novartis, Basel, Switzerland) in children with frequently relapsing nephrotic syndrome; ~50% of patients remained relapse free for 2 years, without serious adverse events. Recently, microemulsified cyclosporine (Neoral, Novartis), which has a more stable absorption profile than conventional cyclosporine, has been developed. We tested the hypothesis that microemulsified cyclosporine is at least as effective as conventional cyclosporine.

Methods. To evaluate the safety and efficacy of microemulsified cyclosporine, a prospective, multicentre trial was conducted according to the previously established protocol, using microemulsified cyclosporine instead of conventional cyclosporine. The duration of treatment was 24 months. During the first 6 months, patients received microemulsified cyclosporine in a dose that maintained the trough level between 80 and 100 ng/mL of cyclosporine. For the next 18 months, the dose was adjusted to maintain a level between 60 and 80 ng/mL.

Results. A total of 62 patients (median age, 5.4 years; 48 males, 14 females) were studied. The frequency of relapse decreased from 4.6 ± 1.4 to 0.7 ± 1.5 times per year (P<0.0001). The probability of relapse-free survival at Month 24 was 58.1% (95% confidence interval, 45.8–70.3%). The probability of progression (to frequently relapsing nephrotic syndrome)-free survival at Month 24 was 88.5% (95% confidence interval, 80.4–96.5%). Cyclosporine nephrotoxicity was detected in only 8.6% of patients who underwent renal biopsy after 2 years of treatment. Antihypertensive agents were administered to 12.9% of the patients to control hypertension without severe sequelae.