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Assessment of serum concentration and urinary excretion of tumor necrosis factor receptor 1 and 2 and their potential as markers of immunoglobulin A nephropathy activity

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Abstract

Background. Tumor necrosis factor receptor 1 (TNFR1) and 2 (TNFR2) can be cleaved from the cell surface and circulate alone or in combination with tumor necrosis factor alpha (TNF-α). These soluble receptors may play a key role in regulating the inflammatory response.

Objectives. The study aimed to evaluate the role of TNFRs in regulating the inflammatory response in immunoglobulin A nephropathy (IgAN).

Materials and methods. The study included 26 patients with newly diagnosed and biopsy-confirmed IgAN and 20 healthy controls. Study material included blood and fresh urine collected the morning before kidney biopsy and therapy. The serum concentrations of TNFR1 (STNFR1) and TNFR2 (STNFR2) and urinary excretion of TNFR1 (UTNFR1) and TNFR2 (UTNFR2) were determined with immunoassay. Subsequently, the data were evaluated statistically.

Results. The STNFR1 and STNFR2 levels were higher in IgAN patients than in healthy subjects (4747.87 pg/mL and 2817.62 pg/mL compared to 2755.68 pg/mL (95% CI: from −2948.41 to −1035.97; $p = 0.001$) and 1437.83 pg/mL (95% CI: from −1958.50 to −419.60; $p = 0.001$). The power of the test was 98.5% for STNFR1 and 96% for STNFR2. Urinary concentrations only increased for TNFR1 (3551.29 compared to 2338.95 pg/mg of creatinine (Cr) (95% CI: from −2247.03 to −177.66; $p = 0.023$). The STNFR1 marker was characterized by a sensitivity of 73.08% and a specificity of 90.00% ($p < 0.001$).

Conclusions. Our results suggest that TNFR1 and TNFR2 are good markers of TNF-α pathway activation in IgAN patients.

Key words: tumor necrosis factor α, tumor necrosis factor receptor I (TNFR1), tumor necrosis factor receptor II (TNFR2), marker of IgA nephropathy

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Background

Tumor necrosis factor alpha (TNF-α) is a pleiotropic cytokine that plays a vital role in inflammatory processes and stimulates the production of cytokines such as interleukin (IL)-1β, IL-6 and IL-8. It also affects the secretion of adhesion molecules at endothelial cell junctions, as well as chemokines such as monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-2 (MIP-2), regulated upon activation, normal T cell expressed and presumably secreted (RANTES), and macrophage inflammatory protein-1 alpha (MIP-1α).[1–3] Furthermore, TNF-α has immunoregulatory properties and can induce several anti-inflammatory and regulatory cytokines.[4] It is mainly produced by macrophages, dendritic cells and T lymphocytes,[5] and is a transmembrane protein (TMP) that can be cleaved[1] by disintegrin and metalloprotease 17 protein (ADAM-17), and released into the circulation as a functional soluble protein.[6] The TNF-α is usually undetectable in healthy kidneys.[4] It binds on the cell surface to 2 transmembrane receptors, tumor necrosis factor receptor 1 (TNFR1) (also known as CD120A or p55) and tumor necrosis factor receptor 2 (TNFR2) (also known as CD120B or P75),[1] which are differently expressed on cells and tissues.

The TNFRs demonstrate various biological effects, including survival, differentiation, proliferation, migration, inflammation, and cell death. The TNFR1 mainly plays a pro-inflammatory role, whereas TNFR2 may be involved in immunoregulation.[5] Both receptors can be cleaved from the cell surface and circulate alone or in combination with TNF-α.[1] The soluble TNFRs can participate in the regulation of inflammatory responses by binding and neutralizing free TNF-α.[4] The TNFR1 can be detected in almost all cell types, while TNFR2 is found in oligodendrocytes, astrocytes, T cells, myocytes, thymocytes, the endothelium, and human mesenchymal stem cells. In the human kidney, TNFR1 is expressed in the normal glomerular endothelium, where it is mainly localized to the Golgi apparatus,[6] whereas TNFR2 is not usually expressed.[4]

Several studies have associated the expression of TNF-α or TNFRs with various kidney diseases.[1] In inflammatory and autoimmune kidney diseases, TNF-α plays a role in the cascade leading to kidney damage, and its expression is associated with damage.[2] Moreover, increased TNFR levels are associated with the progression of various types of glomerulonephritis (GN).[1,7] Monoclonal antibodies can inhibit TNF-α receptor binding.[1] Simultaneous or specific blocking of TNFR1 and TNFR2 may reveal different receptor functions, which, in turn, may prove useful in developing a specific therapeutic strategy targeting TNFR.[2]

Objectives

Immunoglobulin A nephropathy (IgAN) accounts for the highest percentage of GN. Considering the relatively high incidence rate, the foundation of our study was to search for new markers of the disease, and the primary concept was to ensure fast and non-invasive diagnosis of patients with IgAN. The research aimed to assess concentrations of serum TNFR1 (STNFR1), STNFR2, urinary TNFR1 (UTNFR1), and UTNFR2 to determine their potential relationship with clinical markers of IgAN activity.

Materials and methods

Study design, setting and participants

Over a period of 2 years, the study comprised 26 Caucasian patients (15 women and 11 men) with a mean age of 40 ±15 years who presented with newly diagnosed biopsy-confirmed IgAN. The study was conducted in the Department of Nephrology, Transplantology, and Internal Medicine of Poznan University of Medical Sciences. The Bioethics Committee at Poznan University of Medical Sciences reviewed and approved the study (No. 444/11). The control group consisted of 20 healthy individuals, matched for gender and age. All kidney tissue samples were obtained by percutaneous renal biopsy. A standard examination of cortical tissue under light microscopy and immunofluorescence was performed. In all patients, blood and fresh urine were collected in the morning before kidney biopsy and treatment. Urine samples were centrifuged at 1000 g for 5 min, and supernatants were stored at –70°C until tested. The serum concentrations of TNF-α, TNFR1 and TNFR2, and urinary excretion of TNFR1 and TNFR2 were determined using the Quantkine Human soluble TNFRI and soluble TNFRII immunoassay (Cat. No. DRT 100; R&D Systems, Minneapolis, USA). The assay measured soluble TNFRs and TNF-associated soluble TNFRs. The TNFR concentrations were expressed in picograms per milliliter and TNFR excretion was expressed in picograms per milligram of creatinine (Cr).

Variables

We examined renal histological findings using the mesangial hypercellularity, endocapillary hypercellularity, segmental glomerulosclerosis, and tubular atrophy/interstitial fibrosis (MEST) Oxford classification. However, we only analyzed segmental glomerulosclerosis and tubular atrophy/interstitial fibrosis because mesangial hypercellularity (M1 > 0.5) was present in all patients except for one. The severity of tubular atrophy/interstitial fibrosis was classified as T0 (0–25%; n = 14), T1 (26–50%; n = 6) or T2 (>50%; n = 6). Segmental glomerulosclerosis was classified as S0 (absent; n = 7) or S1 (present; n = 19). Unfortunately, we could not assess endocapillary hypercellularity based on pathomorphological descriptions. The estimated glomerular filtration rate (eGFR) was calculated using the Cockcroft–Gault formula. The mean (± standard deviation; M ±SD) eGFR was 97.05 ±24.12 mL/min/1.73 m^2. Normal renal function

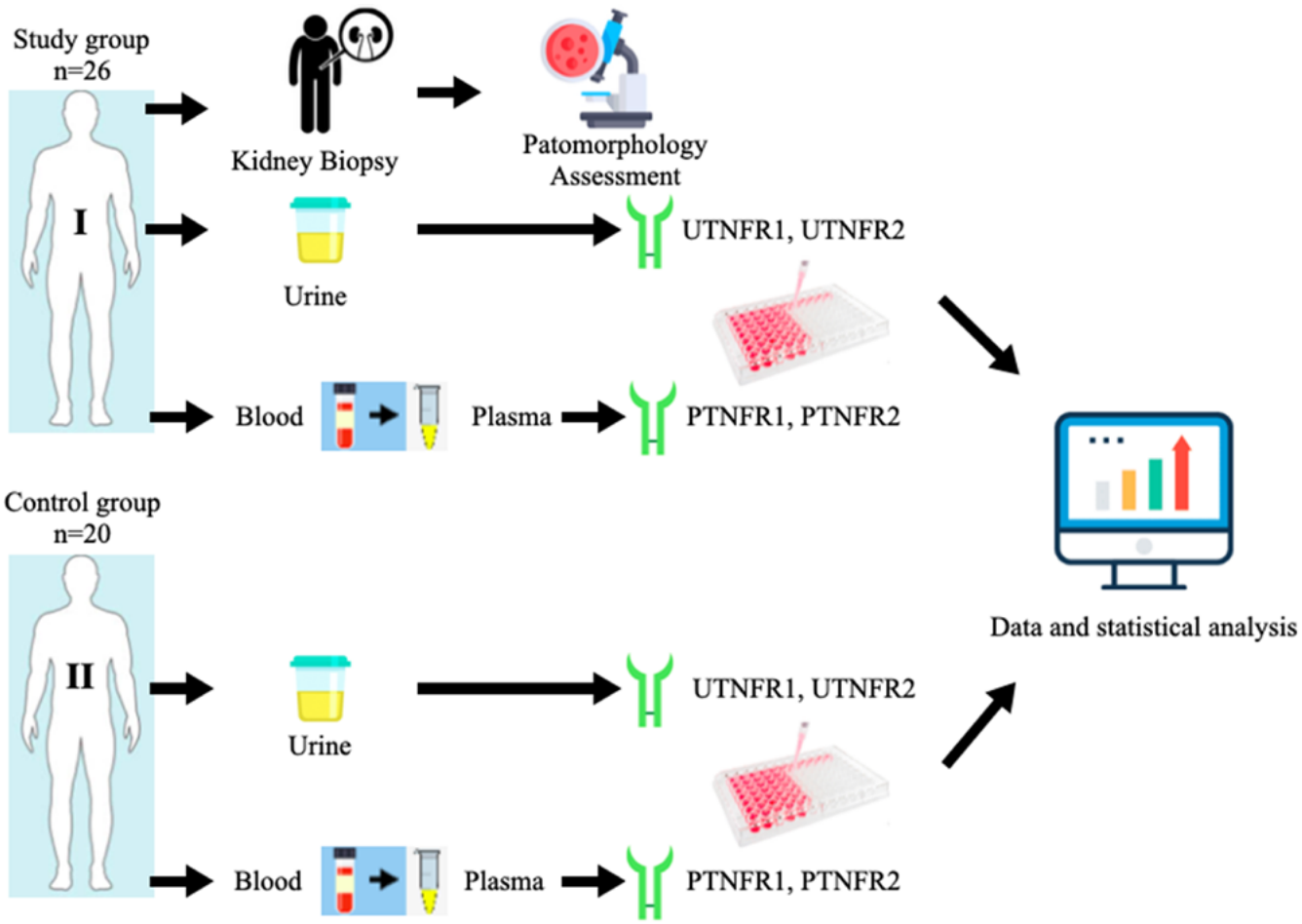

Fig. 1. Materials and methods

was defined as an eGFR ≥90 mL/min/1.73 m^2. The values mentioned above are normal for most IgAN patients. Stage I chronic kidney disease (CKD) was found in 17 subjects, 7 presented stage II, and 2 patients had stage III CKD. Urinary protein excretion (UPE) was measured from 24-hour urine collection, and microscopic analysis of the urine sediment was performed. Eight patients developed nephrotic syndrome, and hypertension (blood pressure ≥140/90 mm Hg) was reported in 6 participants. The characteristics of the research group are presented in Table 1. All patients were treated with an angiotensin-converting enzyme inhibitor (ACEI) and/or an angiotensin II type 1 (AT1) receptor antagonist. Other antihypertensive drugs were also administered to achieve the recommended target blood pressure <130/80 mm Hg or <125/75 mm Hg when urinary protein excretion was >1.0 g/24 h. Fourteen patients were treated with glucocorticoids (GCs), and 2 received GCs and mycophenolate mofetil. The stages of the study are illustrated in Fig. 1.

Bias

The potential effects of non-renal sources on UTNFR1 were reduced by only including subjects without comorbid diseases. As such, patients with symptoms of acute and chronic inflammatory diseases other than GN were excluded from the study. Patients were coded appropriately, and the researcher studying the parameters did not know which group patients were assigned to, though the physician was not blinded.

Study size

The size of the study and control groups was estimated based on a similar study in the available scientific literature.[4]

Statistical analyses

Data are presented as M ±SD and 95% confidence intervals (95% CIs). The correlations between the 2 interval variables were calculated using Pearson's correlation. The Shapiro–Wilk test verified whether the TNFR concentration values followed a normal distribution. The equality of variances was tested using the Fisher–Snedecor test.

Student's t-test compared differences between unpaired variables with a normal distribution and equal variances, and Welch's test compared data with unequal variances. The Mann–Whitney U test assessed differences between unpaired variables with non-normal distribution.

Table 1. Characteristics of the study group

Characteristic		Value	p-value (Shapiro–Wilk test)
Gender (men/women), n		11/15	–
Age [years]		Q1: 27 Q2: 33.5 Q3: 49	0.003
Urinary protein excretion [g/24 h]	patients with nephrotic syndrome (n = 8)	Q1: 4.88 Q2: 8.24 Q3: 12.25	0.017
	patients with non-nephrotic syndrome (n = 18)	1.22 ±0.90	0.115
eGFR [mL/min/1.73 m^2]	patients with eGFR ≥ 90 (n = 17)	109.45 ±18.38	0.057
	patients with eGFR < 90 (n = 9)	73.63 ±14.06	0.240
BP values (systolic/diastolic)	patients with arterial hypertension (n = 6)	142.5 ±6.45/90 ±7.91	0.918/0.505
	normotensive patients (n = 20)	Q1: 115/72.5 Q2: 120/80 Q3: 125/82.5	0.442/0.040

eGFR – estimated glomerular filtration rate; BP – blood pressure. The values are given as number of patients (n) or mean ± standard deviation (M ±SD).

Table 2. Statistical parameters for the assessment of differences in serum concentrations of TNFR1 (STNFR1) and TNFR2 (STNFR2) and urinary excretion of TNFR1 (UTNFR1) and TNFR2 (UTNFR2)

Statistical parameters	STNFR1	STNFR2	UTNFR1	UTNFR2
Shapiro–Wilk test	p = 0.337	**p = 0.039**	p = 0.888	p = 0.072
F test of variance homogeneity	p = 0.675	**p = 0.001**	p = 0.169	p = 0.095
95% CI	**from –2948.41 to –1035.97**	**from –1958.50 to –419.60**	from –2247.03 to –177.66	from –1424.21 to 154.19
t-test for independent groups	**p = 0.001**	**p = 0.001***	**p = 0.023**	p = 0.112
df	44	–	44	44
Power of the test (1-β)×100%	**98.5%**	**96%**	67%	<50%

95% CI – 95% confidence interval; df – degrees of freedom; *Mann–Whitney U test. Values in bold are statistically significant.

A receiver operating characteristic (ROC) curve determined classifier quality, sensitivity and specificity, and the analysis established the optimal cutoff point. All statistical analyses employed MedCalc, v. 20.006 (MedCalc Software Ltd., Ostend, Belgium).

Results

No significant differences were found between serum TNF-α levels of IgAN patients (25.64 pg/mL) and healthy control (26.99 pg/mL) (95% CI: –3.7–5.1; p = 0.627). However, STNFR1 and STNFR2 concentrations were significantly higher in IgAN patients than in healthy participants (4747.87 pg/mL and 2817.62 pg/mL compared to 2755.68 (95% CI: –2948.41––1035.97; p = 0.001) and 1437.83 pg/mL (95% CI:from –1958.50 to –419.60; p = 0.001)). The power of the test was 98.5% for STNFR1 and 96% for STNFR2. The UTNFR1 excretions were considerably higher in IgAN patients compared to healthy subjects (3551.29 pg/mg of Cr compared to 2338.95 pg/mg of Cr (95% CI: from –2247.03 to –177.66; p = 0.023)). The power of the test in this case was 67%. The results are presented in Fig. 2 and Table 2.

Positive correlations were observed between STNFR2 levels in IgAN patients and serum Cr (r = 0.4359, p = 0.026) and urinary protein excretion (r = 0.4639, p = 0.017). Meanwhile, a negative correlation was shown between STNFR2 concentration and serum albumin (r = –0.6392, p = 0.001). The results are illustrated in Fig. 3.

The marker quality was assessed using the ROC analysis, and their sensitivity and specificity were described accordingly. The STNFR1 showed a sensitivity of 73.08% and a specificity of 90.00% (p < 0.001) (Fig. 4), with the optimal cutoff point established at 3381 pg/mL. The other results of the analysis are presented in Table 3.

In patients with nephrotic syndrome, STNFR2 level was significantly higher compared to patients with non-nephrotic proteinuria (3904.55 pg/mL compared to 2334.53 pg/mL; 95% CI: 261.15–2878.88; p = 0.021), although no significant differences were detected between these 2 groups for STNFR1 (4521.44 pg/mL compared to 4848.50 pg/mL; 95% CI: from –1815.30 to 1635.00; p = 0.567), UTNFR1 (3102.55 pg/mg of Cr compared to 3750.73 pg/mg of Cr; 95% CI: from –2353.61 to 1057.24; p = 0.441) or UTNFR2 (2268.14 pg/mg of Cr compared to 2713.76 pg/mg of Cr; 95% CI: –1774.57–883.32; p = 0.496).

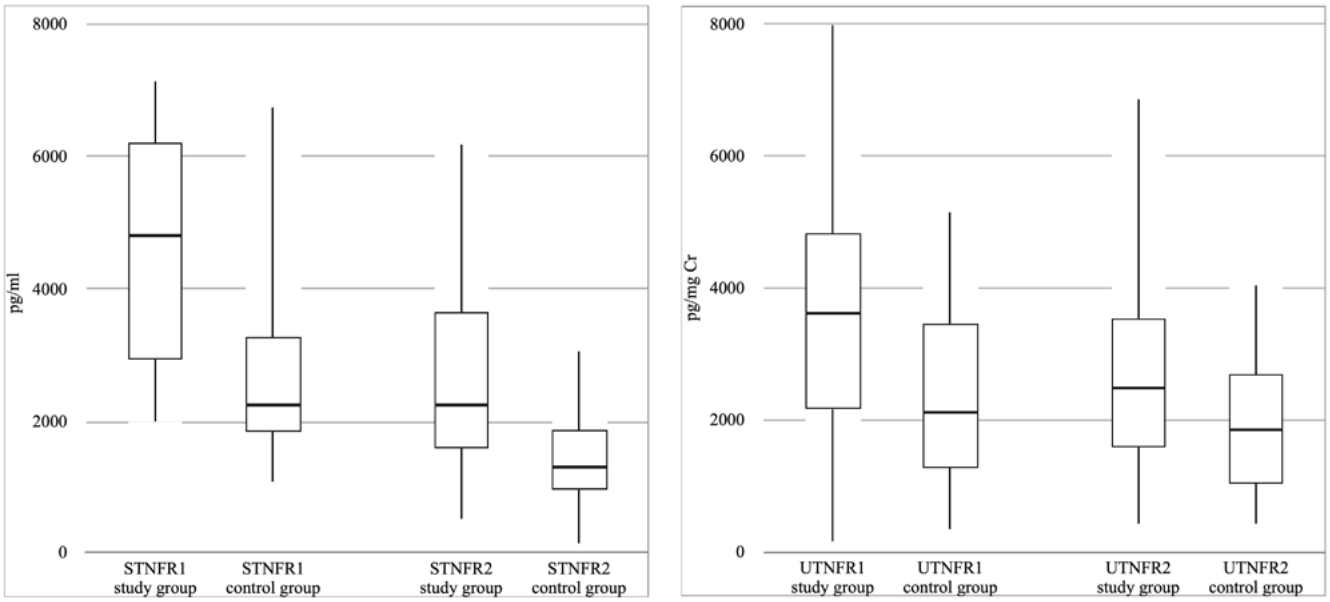

Fig. 2. Comparison of mean serum tumor necrosis factor receptor 1 (STNFR1), STNFR2, urinary TNFR1 (UTNFR1), and UTNFR2 levels in immunoglobulin A nephropathy (IgAN) patients and healthy controls

Table 3. Receiver operating characteristic (ROC) curve

Variable	Sensitivity	Specificity	Values	Youden's J statistic	AUC	Standard error	p-value
STNFR1	73.08	90.00	3381 pg/mL	0.6308	0.813	0.0685	**<0.001**
STNFR2	76.92	70.00	1561.4 pg/mL	0.4692	0.783	0.0683	**<0.001**
UTNFR1	69.23	70.00	2407.1 pg/mg Cr	0.3923	0.698	0.0786	**0.012**
UTNFR2	50.00	75.00	2474.7 pg/mg Cr	0.2500	0.631	0.0831	0.116

STNFR1 – serum concentrations of tumor necrosis factor receptor 1 (TNFR1); STNFR2 – serum concentrations of TNFR2; UTNFR1 – urinary excretion of TNFR1; UTNFR2 – urinary excretion of TNFR2; Cr – serum creatinine; AUC – area under the ROC curve. Values in bold are statistically significant.

The patients with an eGFR < 90 mL/min/1.73 m^2 had significantly higher STNFR2 values than patients with an eGFR ≥ 90 mL/min/1.73 m^2 (3809.51 pg/mL compared to 2292.49 pg/mL; 95% CI: 245.93–2788.10; p = 0.021). No significant differences were observed in STNFR1 (5016.70 pg/mL compared to 4605.54 pg/mL; 95% CI: –1019.07–1841.39; p = 0.559), UTNFR1 (3904.10 pg/mg of Cr compared to 3364.51 pg/mg of Cr; 95% CI: from –1120.50 to 2199.68; p = 0.509) or UTNFR2 (3170.61 pg/mg of Cr compared to 2262.19 pg/mg of Cr; 95% CI: from –336.13 to 2152.97; p = 0.145).

Comparisons of patients with arterial hypertension (blood pressure ≥140/90 mm Hg) to patients without hypertension revealed no significant differences in levels of STNFR1 (4606.78 pg/mL compared to 4790.19 pg/mL; 95% CI: from –1808.33 to 1441.51; p = 0.818), STNFR2 (2417.53 pg/mL compared to 2937.64 pg/mL; 95% CI: from –2111.57 to 1071.35; p = 0.506), UTNFR1 (4071.82 pg/mg of Cr compared to 3395.14 pg/mg of Cr; 95% CI: from –2466.10 to 3819.46; p = 0.617), and UTNFR2 (3719.53 pg/mg of Cr compared to 2233.78 pg/mg of Cr; 95% CI: from –757.48 to 3728.99; p = 0.156).

There were no differences between patients for mean STNFR1, STNFR2, UTNFR1, or UTNFR2 values based on segmental glomerulosclerosis or tubular atrophy/interstitial fibrosis severity (Table 4).

Discussion

Immunoglobulin A nephropathy is a form of glomerulopathy known more specifically as GN and represents the most common primary GN in many countries. The disease can be classified into histological and clinical types, though the pathogenetic mechanisms are not entirely known. However, mesangial pathogenic polymeric IgA1 (galactose-deficient IgA1; Gd-IgA1) deposition, mesangial cell proliferation, increased extracellular matrix synthesis, and glomerular infiltration of macrophages, monocytes and T lymphocytes are frequently observed. The unique localization is due to the presence of IgA1 (CD71) receptors on mesangial cells.[8,9] Immunoglobulin A initiates an immune reaction and combines with the resulting antibodies to form immune complexes that accumulate as deposits in the mesangium, which leads to cellular and mesangial matrix proliferation.

Clinically, the first manifestation of IgAN is an episode of hematuria, which predominantly occurs following

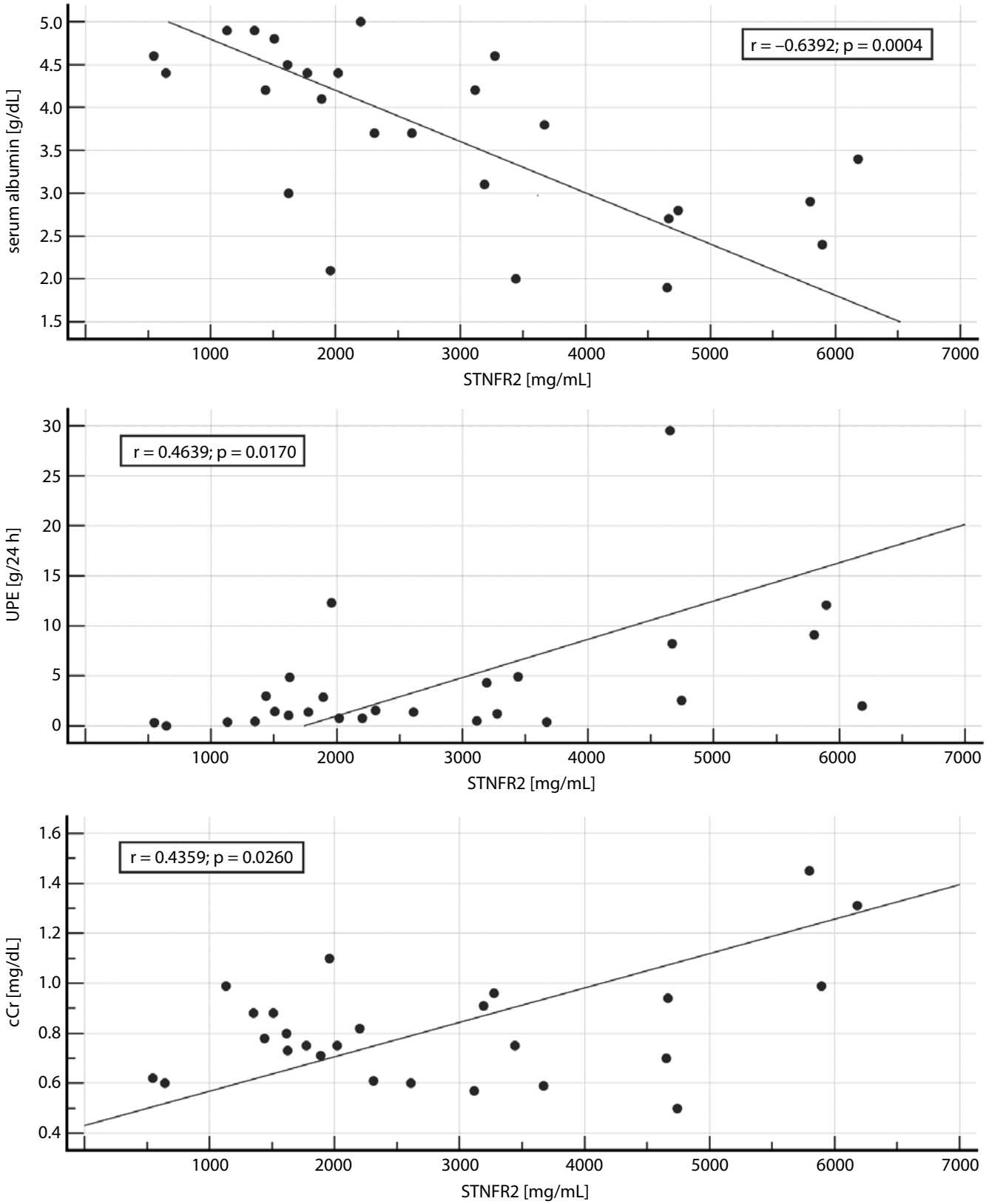

Fig. 3. The correlation (Pearson's correlation) between serum tumor necrosis factor receptor 2 (STNFR2), serum creatinine (Cr), urinary protein excretion (UPE), and serum albumin in patients with primary immunoglobulin A nephropathy (IgAN)

a non-specific upper respiratory tract infection and resolves spontaneously after several days. The picture may vary in the pathomorphological examination. Therefore, an additional immunofluorescence test should be performed.[8] Evidence suggests that cytokines play a crucial role in IgAN pathogenesis and progression, with TNF-α having a notable involvement. Indeed, TNF-α expression is preferentially increased in the glomeruli in various

Table 4. The relationship between histopathological kidney examination and tumor necrosis factor receptor (TNFR) concentrations

Statistical parameter	STNFR1	STNFR2	UTNFR1	UTNFR2
Shapiro–Wilk test	p = 0.253	p = 0.077	p = 0.941	p = 0.607
F test of variance homogeneity	p = 0.967	p = 0.832	p = 0.729	p = 0.290
S0	5585.38	2859.64	3732.90	1819.00
S1	4570.11	2931.96	4153.06	3123.11
p-value of t-test for independent groups	p = 0.246	p = 0.931	p = 0.655	p = 0.103
df	19	19	19	19
Shapiro–Wilk test	p = 0.140	p = 0.078	p = 0.732	p = 0.390
F test of variance homogeneity	p = 0.669	p = 0.556	p = 0.094	p = 0.350
T0	4962.58	2602.55	4493.71	3073.48
T1	4669.53	2637.65	4444.70	2793.60
p-value of t-test for independent groups	p = 0.766	p = 0.969	p = 0.959	p = 0.768
df	14	14	14	14
Shapiro–Wilk test	**p = 0.037**	**p = 0.042**	p = 0.494	p = 0.333
F test of variance homogeneity	p = 0.664	p = 0.739	p = 0.822	p = 0.872
T0	4962.58	2602.55	4493.71	3073.48
T2	4563.92	3885.66	2682.02	2201.70
p-value of t-test for independent groups	p = 0.646*	p = 0.104*	p = 0.080	p = 0.348
df	–	–	15	15

UTNFR1 – urinary excretion of TNFR1; UTNFR2 – urinary excretion of TNFR2; STNFR 1 – serum concentration of TNFR1; STNFR 2 – serum concentration of TNFR2; df – degrees of freedom; *Mann–Whitney U test. Values in bold are statistically significant.

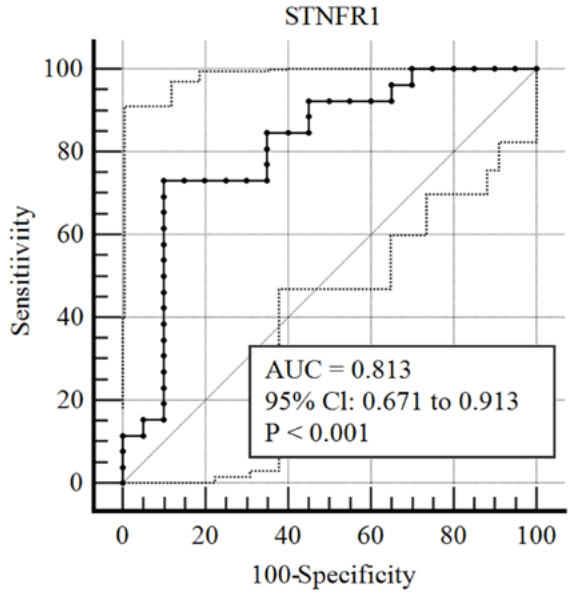

Fig. 4. Receiver operating characteristic (ROC) curve chart (black dotted line) and its 95% CI (95% confidence intervals) for serum tumor necrosis factor receptor 1 (STNFR1)

AUC – area under the ROC curve.

forms of GN,[4,10,11] and correlates with increased serum and urinary excretion of the cytokine.[4] These observations indicate that damaged kidneys constitute the source of increased production and excretion of TNF-α and TNFRs in primary GN patients. Moreover, TNFR1 and TNFR2 are involved in the caspase activation pathways after binding to TNF-α and cause epithelial cell proliferation in the proximal tubule and renal interstitial damage. Interestingly, both receptors can also be cleaved from the cell surface, circulate alone or in combination with TNF-α, and may participate in inflammatory response regulation.

There is a strong correlation between tubulointerstitial tissue damage severity and subsequent renal function deterioration in IgAN and diabetic nephropathy (DN).[12] Chan et al. demonstrated that after IgA deposition, TNF-α released from mesangial cells activates proximal tubular epithelial cells (PTEC), leading to further inflammatory changes in the renal interstitium.[12] In contrast, Lai et al. found that IgA-conditioned medium prepared by culturing human mesangial cells (IgA-HMC medium) with IgA from IgAN patients significantly increased gene expression and TNF-α synthesis by podocytes. These, in turn, may play a role in interstitial damage development in IgAN by enhancing tubular epithelial cell activation and increasing TNF-α synthesis in mesangial cell inflammatory lesion cultures. However, TNF-α from mesangial cells and podocytes increases TNFR expression.[9] This mechanism leads to increased TNFR excretion from mesangial cell and podocyte membranes, which may account for the increased serum and urine TNFR levels observed in our IgAN patients.

Several studies have found that levels of the molecules associated with circulating TNF pathway markers, such as TNF-α and TNFRs, are significantly higher in CKD patients and that these levels correlate closely with changes in eGFR.[4,13–15] Moreover, the results of the Joslin Kidney Study demonstrated that increased levels of circulating

TNFR1 and TNFR2 were very strong predictors of DN progression to end-stage renal disease (ESRD).[16,17] However, little is known about the clinical and histological association of circulating TNFRs, or the urinary excretion of TNFRs, in IgAN patients.

Our previous study showed that UTNFR1 excretion was significantly higher in patients with various types of chronic GN, including approx. 35% of IgAN patients, compared to healthy controls.[4] Although Sonoda et al. did not find differences in UTNFR levels between IgAN patients and healthy controls, they observed that STNFR levels were considerably higher in IgAN patients.[14] It should be emphasized that STNFR measurements were not performed in our previous study. The present study confirmed that TNFR1 and TNFR2 serum levels were significantly higher in patients with IgAN than in healthy subjects. However, urinary excretion was only significantly higher for TNFR1. These results indicate that STNFR1, STNFR2 and UTNFR1 levels may constitute a marker of the TNF-α pathway activation in the kidneys, which contributes to the deterioration of renal function. Moreover, TNF-α pathway activation directly increases glomerular vasoconstriction and albumin permeability, while kidney exposure to TNF-α increases TNFR messenger ribonucleic acid (mRNA) expression in the renal interstitial tubule, causing cell death.

Zwiech et al. observed a significant increase in STNFR1, STNFR2, UTNFR1, and UTNFR2 in their study group (patients with IgAN).[15] In our previous paper, UTNFR excretion correlated negatively with eGFR in IgAN patients.[4] Sonoda et al. also emphasized the negative correlation between serum and urinary excretion of TNFRs with eGFR in IgAN patients, although the correlation between STNFRs and eGFR was stronger than the correlation between UTNFRs and eGFR.[14] In the present study, a negative correlation was also observed between serum and urinary excretion of TNFRs and eGFR in IgAN patients. However, the correlation between UTNFRs and eGFR was stronger than between STNFRs and eGFR (STNFR1 ($r = -0.1060$, $p = 0.606$), STNFR2 ($r = -0.0899$, $p = 0.663$), UTNFR1 ($r = -0.1547$, $p = 0.451$), UTNFR2 ($r = -0.3466$, $p = 0.083$)). These data suggest that increased STNFR and UTNFR levels confirm the activation of the renal TNF-α pathway in IgAN patients.

There was no significant correlation between UTNFRs and STNFRs. Conversely, experimental studies have demonstrated that the induction of immune damage to the kidneys (anti-glomerular basement membrane antibody-induced experimental nephritis) results in higher STNFR1 and UTNFR1 values.[18]

Each TNFR plays a separate role in inflammation, apoptosis and necrosis. However, the current study found a strong correlation between urinary excretion of TNFR1 and TNFR2 (Pearson's correlation coefficient, $r = 0.8218$, $p < 0.001$), but no correlation was observed between serum TNFR1 and TNFR2 (Pearson's correlation coefficient, $r = 0.1146$, $p = 0.577$). Another vital issue is the source of TNFRs in serum and urine. The TNFR levels were compared in patients who presented with severe tubulointerstitial fibrosis on histopathology, with the group showing either no or mild tubulointerstitial fibrosis. However, no significant differences were noted for STNFR concentrations and UTNFR excretion. In addition, TNFR levels were compared in patients with and without nephrotic syndrome, and no relevant results were obtained.

A significant positive correlation was found between STNFR2, serum Cr ($r = 0.4359$, $p = 0.026$) and UPE ($r = 0.4407$, $p = 0.027$). Nevertheless, a negative correlation was observed between the STNFR2 concentrations and serum albumin ($r = -0.6392$, $p = 0.001$). Urinary protein excretion is considered an indicator of the severity of glomerular barrier damage, and a critical factor contributing to tubulointerstitial tissue damage and accelerated fibrotic processes, which lead to a rapid deterioration in kidney function.

In the available scientific literature, no study has assessed the applicability of TNFR concentrations in serum and urine as potential markers of IgAN. The marker quality was evaluated using ROC analysis, and sensitivity and specificity of TNFR concentrations were described. The potential marker, STNFR1, characterized by a sensitivity of 73.08% and a specificity of 90%, was statistically significant ($p < 0.001$). The optimal cutoff point for TNFR1 was determined to be 3381 pg/mL. Given that the power of the test for differences in STNFR1 concentrations between the control and the study groups was 98.5%, we can conclude that STNFR1 may prove to be a good marker of IgAN activity.

Limitations

The study was limited due to a lack of immunohistochemical tests, meaning that evaluating TNFR expression in kidney biopsy samples was not possible. Therefore, increased TNFR expression in renal tissue could not be directly determined. The causal relationship between TNFRs and renal tubulointerstitial fibrosis remains unclear due to the cross-sectional design. The 2nd limitation was the low number of patients and the lack of a follow-up study estimating the correlation of initial STNFR and UTNFR concentrations with disease course and progression.

The study aimed to assess TNFR concentrations in serum and urine. However, these parameters were not examined in other types of GN due to difficulties in obtaining a sufficiently large study group. Nonetheless, IgAN is the most common type of GN, and disease incidence is lower for other types of primary GN. As such, time is needed to gather enough patients to compare our results with other GN types. Future studies should account for this aspect and explore it further to obtain information on which types of GN have elevated levels of the markers studied.

Conclusions

The results presented above indicate that significantly increased STNFR1, STNFR2 and UTNFR1 concentration levels may be considered good markers of renal TNF-α pathway activation in patients with newly diagnosed IgAN. The assessment of STNFR2 concentration may represent an effective instrument for estimating IgAN activity, and should be considered in clinical practice. Finally, further studies should focus on the correlations between histopathological changes and the applicability of TNFR2 as a non-invasive marker of IgAN prognosis.

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References

1. McAdoo SP, Pusey CD. Is there a role for TNFα blockade in ANCA-associated vasculitis and glomerulonephritis? *Nephrol Dial Transplant*. 2017;32(Suppl 1):i80–i88. doi:10.1093/ndt/gfw361
2. Merkle M, Ribeiro A, Wörnle M. TLR3-dependent regulation of cytokines in human mesangial cells: A novel role for IP-10 and TNF-α in hepatitis C-associated glomerulonephritis. *Am J Physiol Renal Physiol*. 2011;301(1):F57–F69. doi:10.1152/ajprenal.00083.2011
3. Upadhyay A, Larson MG, Guo CY, et al. Inflammation, kidney function and albuminuria in the Framingham Offspring cohort. *Nephrol Dial Transplant*. 2011;26(3):920–926. doi:10.1093/ndt/gfq471
4. Idasiak-Piechocka I, Oko A, Pawliczak E, Kaczmarek E, Czekalski S. 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. *Nephrol Dial Transplant*. 2010;25(12):3948–3956. doi:10.1093/ndt/gfq310
5. Huang YS, Fu SH, Lu KC, et al. Inhibition of tumor necrosis factor signaling attenuates renal immune cell infiltration in experimental membranous nephropathy. *Oncotarget*. 2017;8(67):111631–111641. doi:10.18632/oncotarget.22881
6. Speeckaert MM, Speeckaert R, Laute M, Vanholder R, Delanghe JR. Tumor necrosis factor receptors: Biology and therapeutic potential in kidney diseases. *Am J Nephrol*. 2012;36(3):261–270. doi:10.1159/000342333
7. Wu T, Xie C, Wang HW, et al. Elevated urinary VCAM-1, P-selectin, soluble TNF receptor-1, and CXC chemokine ligand 16 in multiple murine lupus strains and human lupus nephritis. *J Immunol*. 2007;179(10):7166–7175. doi:10.4049/jimmunol.179.10.7166
8. Shu KH, Lee SH, Cheng CH, Wu MJ, Lian JD. Impact of interleukin-1 receptor antagonist and tumor necrosis factor-α gene polymorphism on IgA nephropathy. *Kidney Int*. 2000;58(2):783–789. doi:10.1046/j.1523-1755.2000.00227.x
9. Lai KN, Leung JCK, Chan LYY, et al. Activation of podocytes by mesangial-derived TNF-α: Glomerulo-podocytic communication in IgA nephropathy. *Am J Physiol Renal Physiol*. 2008;294(4):F945–F955. doi:10.1152/ajprenal.00423.2007
10. Navarro JF, Mora C, Gomez M, Muros M, Lopez-Aguilar C, Garcia J. Influence of renal involvement on peripheral blood mononuclear cell expression behaviour of tumour necrosis factor- and interleukin-6 in type 2 diabetic patients. *Nephrol Dial Transplant*. 2007;23(3):919–926. doi:10.1093/ndt/gfm674
11. Navarro JF, Mora C, Muros M, Garcia J. Urinary tumour necrosis factor-excretion independently correlates with clinical markers of glomerular and tubulointerstitial injury in type 2 diabetic patients. *Nephrol Dial Transplant*. 2006;21(12):3428–3434. doi:10.1093/ndt/gfl469
12. Chan LYY, Leung JCK, Tsang AWL, Tang SCW, Neng Lai K. Activation of tubular epithelial cells by mesangial-derived TNF-α: Glomerulotubular communication in IgA nephropathy. *Kidney Int*. 2005;67(2):602–612. doi:10.1111/j.1523-1755.2005.67116.x
13. Kacprzyk F. Serum concentration and urinary excretion of soluble receptors for tumor necrosis factor in patients with primary glomerulonephritis [in Polish]. *Pol Merkur Lek*. 2003;15(88):383–386; discussion 386–387. PMID:14974372.
14. Sonoda Y, Gohda T, Suzuki Y, et al. Circulating TNF receptors 1 and 2 are associated with the severity of renal interstitial fibrosis in IgA nephropathy. *PLoS One*. 2015;10(4):e0122212. doi:10.1371/journal.pone.0122212
15. Zwiech R, Kacprzyk F, Szuflet A, Nowicki M. Prognostic values of serum concentration and urinary excretion of interleukin-1 receptor antagonist and tumor necrosis factor receptors type I and II in patients with IgA nephropathy [in Polish]. *Pol Arch Med Wewn*. 2005;113(4):326–333. PMID:16209246.
16. Gohda T, Niewczas MA, Ficociello LH, et al. Circulating TNF receptors 1 and 2 predict stage 3 CKD in type 1 diabetes. *J Am Soc Nephrol*. 2012;23(3):516–524. doi:10.1681/ASN.2011060628
17. Niewczas MA, Gohda T, Skupien J, et al. Circulating TNF receptors 1 and 2 predict ESRD in type 2 diabetes. *J Am Soc Nephrol*. 2012;23(3):507–515. doi:10.1681/ASN.2011060627
18. Wu T, Xie C, Bhaskarabhatla M, et al. Excreted urinary mediators in an animal model of experimental immune nephritis with potential pathogenic significance. *Arthritis Rheum*. 2007;56(3):949–959. doi:10.1002/art.22556