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DIAGNOSTIC SIGNIFICANCE OF TGF-β1 IN KIDNEY RECIPIENTS WITH GRAFT DYSFUNCTION

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Development of minimally invasive diagnosis techniques for complications in recipients, based on analysis of the levels of molecular and genetic biomarkers, is an urgent task facing modern transplantology. Transforming growth factor beta 1 (TGF-β1), which has multiple effects in the body, among the potential indicators of complications. **Objective:** to assess the diagnostic significance of serum TGF-β1 in kidney recipients with graft dysfunction. **Materials and methods.** The study included 129 kidney recipients aged 17 to 68 years and 35 healthy subjects. Serum TGF-β1 levels in the recipients were determined by immunoenzyme technique. **Results.** Kidney recipients included 95 patients with laboratory and clinical signs of graft dysfunction, who underwent biopsy of the transplanted kidney, followed by morphological examination, and 34 recipients with normal graft function. Serum TGF-β1 levels in the kidney recipients were significantly higher than in their healthy counterparts ($p = 0.00001$); it did not correlate with most blood test parameters; with the glomerular filtration rate (GFR). Kidney recipients with graft dysfunction had significantly higher TGF-β1 levels than other recipients ($p = 0.018$). In recipients with graft dysfunction, morphological study revealed the following: acute tubular necrosis (ATN, n = 11), acute T-cell mediated rejection (ACR, n = 26), acute antibody-mediated rejection (AMR, n = 35), non-immune-mediated nephrosclerosis with signs of calcineurin inhibitor nephrotoxicity (CNI nephrotoxicity, n = 13), and recurrent glomerulonephritis (chronic graft rejection, n = 10). Recipients with immune-mediated graft injury (ACR, AMR and chronic rejection) had higher serum TGF-β1 levels than recipients with graft dysfunction resulting from other causes, $p < 0.0001$. Kidney recipients with serum TGF-β1 levels above the threshold value of 94.3 ng/mL had a higher risk of immune-mediated graft dysfunction than other kidney recipients (RR = 2.2 ± 0.22 [95% CI 1.46–3.46]) with 77.5% test sensitivity and 60.3% specificity. **Conclusion.** The calculated threshold serum TGF-β1 level in kidney recipients can be considered as an auxiliary indicator of graft dysfunction resulting from acute or chronic rejection.

Keywords: transforming growth factor beta, TGF-β1, kidney transplantation, graft dysfunction, diagnosis.

INTRODUCTION

Chronic kidney disease (CKD) has a high prevalence worldwide and is among the leading diseases with profound socioeconomic consequences [1]. Kidney transplantation (KT) is a radical and the most effective treatment for CKD [2].

Despite the high efficiency of KT, the risk of kidney graft injury and dysfunction persists throughout subsequent life. An objective method of verifying the pathology of a transplanted organ is biopsy, which is associated with all the limitations and risks of invasive interventions. The development of the concept of personalized methods of minimally invasive diagnosis of complications in the posttransplant period based on the analysis of the levels of molecular and genetic biomarkers and their combinations seems to be an urgent task [3]. Despite the obvious expediency of analyzing biomarkers in the urine of kidney recipients, such tests have not shown sufficient reliability for differentiating the processes of extracellular matrix accumulation associated with chronic rejection.

The list of potential biomarkers of kidney graft injury is constantly expanding and includes representatives of microRNA families, cell-free DNA, protein molecules, etc. [4]. There is a constant search for organ-specific biomarkers that signal not only the development of pathology of the transplanted kidney, but also the nature or degree of damage to the organ.

TGF-β1, which has multiple effects – it is involved in the regulation of immune response, has anti-inflammatory and immunosuppressive effects, and is involved in the synthesis of extracellular matrix proteins [5]. TGF-β1 is a cytokine that promotes collagen production by fib-

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roblasts with subsequent structural changes in the graft and the development of dysfunction [6].

Serum TGF-β1 level was found to be associated with liver fibrosis in children with congenital hepatobiliary abnormalities, and is also associated with the severity of liver, kidney, and heart graft fibrosis [7].

Assessment of serum TGF-β1 level in kidney recipients can be of practical implications for optimizing the diagnosis of complications in kidney recipients.

The aim of this work was to evaluate the diagnostic significance of serum TGF-β1 levels in kidney recipients with graft dysfunction.

MATERIALS AND METHODS

The study included 129 adult kidney recipients who underwent allotransplantation from a related kidney allotransplantation (RKAT) or cadaveric kidney allotransplantation (CKAT) in the period from 1999 to 2022 at Shumakov National Medical Research Center of Transplantology and Artificial Organs. The number of selected recipients included 95 with signs of graft dysfunction that required unscheduled punch biopsy and 34 without signs of graft dysfunction. The dysfunction criteria were elevated creatinine and urea levels and proteinuria. The comparison group consisted of 35 healthy individuals, selected randomly and not differing significantly in terms of age and gender from the recipients. In accordance with the patient management protocol at Shumakov National Medical Research Center of Transplantology and Artificial Organs and the clinical guidelines of the Russian Transplant Society, all recipients after KT underwent routine examinations, which included a clinical assessment of the condition, full blood count and blood chemistry tests with determination of tacrolimus levels, and graft biopsy.

Serum TGF-β1 levels were measured. Blood samples were collected in disposable tubes, centrifuged, serum was frozen and stored at –20 °C. The serum concentration of the biomarker was measured by enzyme immunoassay using specific reagent kits Human TGF-beta 1 ELISA Kit (RayBio®, USA) according to instructions. Blood samples were collected for analysis of TGF-β1 levels on the day of biopsy and other routine laboratory tests (full blood count, blood chemistry test, special blood test).

The pathology was verified via morphological studies of biopsy material. Graft glomerular filtration rate (GFR) was calculated using the CKD-EPI formula, which considers race, sex, age and serum creatinine level.

For comparative analysis of independent variables, nonparametric statistics methods – Mann–Whitney U test and Spearman correlation test – were used. Group differences were considered significant at $p < 0.05$. ROC analysis was used to determine the diagnostic significance of the biomarker and its threshold level. The main diagnostic characteristics of the test were evaluated: relative risk (RR), 95% CI limits, sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic efficiency. Statistical data processing was performed using the Statistica v.13.0 program package, StatSoftInc (USA).

RESULTS

The study included 129 kidney recipients aged 17 to 68 years, including 62 (48%) males and 67 (52%) females.

The major proportion of patients (78%) underwent CKAT and the remaining 22% underwent RKAT. The follow-up period for the recipients ranged from 2 to 4748 days (median, 345 days); 76% of the patients were examined in the long term (>1 month since transplantation). The main characteristics of the recipient group are presented in Table 1.

Serum TGF-β1 levels in the individuals included in the study varied widely, 92.38 [31.77; 129.70] ng/mL, did not differ significantly between men and women ($p = 0.37$), and did not correlate with age ($r = 0.09$; $p = 0.18$).

TGF-β1 levels in kidney recipients were significantly different and higher than in healthy individuals, $p = 0.00001$. A comparative analysis of TGF-β1 concentration in RKAT and CKAT groups showed no significant differences ($p = 0.32$).

There was no significant correlation between TGF-β1 level and length of time (days) since transplantation ($r = 0.137$; $p = 0.13$); there were no significant differences in TGF-β1 concentration in kidney recipients at early (<30 days) and late (>30 days) periods after transplantation ($p = 0.47$).

The relationship between TGF-β1 levels and the main indicators of full blood count, blood chemistry test, special blood test and urinalysis was studied (Table 2).

Correlation analysis showed that TGF-β1 level have no association with most blood test parameters, as well as with graft GFR, but there was a positive correlation with platelet count ($r = 0.206$; $p = 0.025$) and a negative correlation with aspartate aminotransferase (AST) activity ($r = –0.213$; $p = 0.024$). TGF-β1 levels were independent of recipients' serum tacrolimus level.

Evaluation of the association of serum TGF-β1 levels with urinalysis parameters showed a strong positive correlation with red blood cell count ($r = 0.354$; $p = 0.00001$), white blood cell count ($r = 0.245$; $p = 0.006$) and proteinuria ($r = –0.280$; $p = 0.001$).

Of all 129 patients included in the study, 95 patients were categorized as "with graft dysfunction" and 34 were designated as "normal function" recipients based on laboratory and clinical data. Graft function scores in both groups are shown in Table 3.

Kidney recipients with graft dysfunction had significantly higher levels of creatinine and urea, GFR and proteinuria ($p < 0.00001$) than those without. Comparati-

ve analysis of serum TGF-β1 levels in these groups also showed significant differences (p = 0.0004).

Based on the results of morphological study of biopsy specimens from recipients with graft dysfunction, the following pathology variants were identified: ATN (n = 11) in the early post-transplant period, ACR (n = 26), AMR (n = 35), non-immune response interstitial fibrosis with signs of calcineurin inhibitor nephrotoxicity (CNI nephrotoxicity, n = 13), recurrent glomerulonephritis (chronic graft rejection, n = 10). See Fig. 1.

A comparative analysis showed significantly higher TGF-β1 levels in recipients with ACR (p = 0.0003), AMR (p = 0.002) and chronic rejection (p = 0.001) compared to recipients without dysfunction (Fig. 2).

Table 1

Basic characteristics of kidney recipients and healthy subjects included in the study

Indicator		Kidney recipients	Healthy individuals
Number, n		129	35
Gender, n (%):	Male Female	62 (48%) 67 (52%)	18 (52%) 17 (48%)
Age, years:	Range Median [Interquartile range]	17 to 68 40 [33; 51]	21 to 64 38 [26; 50]
Type of transplantation, n (%):	Deceased donor (CKAT) Living-related donor (RKAT)	101 (78%) 28 (22%)	–
Graft function, n (%):	Normal function Signs of graft dysfunction	34 (26%) 95 (74%)	–
Follow-up period, days:	Range; Median [Interquartile range]	2 to 4748 325 [39; 1448]	–
Post-transplant period, n (%):	Early (≤1 month) Late (>1 month)	31 (24%) 98 (76%)	–
TGF-β1 level, ng/mL:	Median [Interquartile range]	104.0 [79.10; 138.80]	6.66 [3.87; 17.45]

Table 2

Correlation of TGF-β1 levels with full blood count, biochemical tests and urinalysis indicators in kidney recipients

Indicator	Spearman's rank correlation (r)	Significance level (p)
Full blood count		
Hemoglobin (g/L)	0.037	0.689
White blood cells (10^9/L)	0.075	0.496
Platelets (10^9/L)	0.206	0.025
Blood chemistry test		
Total protein (g/L)	–0.115	0.234
Urea (mmol/L)	0.111	0.219
Creatinine (μmol/L)	0.121	0.179
ALT (U/L)	–0.095	0.355
AST (U/L)	–0.246	0.015
Glucose (mmol/L)	0.102	0.308
Special blood test		
GFR (mL/min/1.73 m^2)	–0.026	0.76
Tacrolimus (ng/mL)	–0.044	0.630
Urinalysis		
Red blood cells (in the field of view)	0.354	0.00001
Leukocytes (in the field of view)	0.245	0.006
Proteinuria (g/L)	0.280	0.001

There were no significant differences in TGF-β1 levels in ATN or CNI nephrotoxicity compared to recipients with normal graft function (p = 0.82 and p = 0.36, respectively).

Kidney recipients with ACR, AMR and chronic rejection, in which immune processes play a leading role in their development, were grouped under "immune mechanisms" of graft injury. The ATN and CNI nephrotoxicity group constituted the group with graft dysfunction, labeled as "other processes". A comparative analysis of TGF-β1 levels and basic laboratory parameters of kidney function in recipients with normal graft function and dysfunction resulting from immune (ACR, AMR, chronic rejection) and other processes (ATN, CNI nephrotoxicity) was conducted.

In recipients with graft dysfunction resulting from immune mechanisms, TGF-β1 levels were not only significantly different from those in recipients with normal function (p < 0.000) but were also higher than in dysfunction caused by other processes (p = 0.0007; Fig. 3).

At the same time, the level of classical renal function parameters (creatinine, urea, proteinuria and GFR) did not differ significantly between recipients with immune and non-immune injury.

Based on the results obtained, the diagnostic significance of TGF-β1 level for identifying recipients with graft dysfunction resulting from immune mechanisms (ACR, AMR, chronic rejection) was assessed. The area under the ROC curve was 0.721 ± 0.04 [95% CI 0.64–0.80] and was significantly different from 0.5, $p < 0.001$ (Fig. 4).

The threshold serum TGF-β1 level for detection of kidney graft dysfunction arising from acute and chronic rejection mechanisms was 94.3 ng/mL. Kidney recipients with TGF-β1 levels exceeding this calculated threshold had a 2.2-fold higher risk of acute or chronic graft rejection resulting from immune mechanisms detected on morphological examination than other kidney recipients (RR = 2.2 ± 0.22 [95% CI 1.46–3.46] with 77.5% sensitivity, 60.3% specificity, and 70.0% overall diagnostic performance of the test). The positive and negative predictive values of serum TGF-β1 measurements for identifying patients at high risk of immunological complications after KT was 70.5% and 68.6%, respectively.

DISCUSSION

Active research in recent years in the field of biochemistry, immunology and genetics has not only expanded the understanding of the complex mechanisms of interaction between the recipient body and the donor organ, but also opened up additional opportunities for the development of innovative approaches to improving and predicting transplant outcomes. The mechanisms of tolerance and rejection of a transplanted organ include a whole set of complex immune processes [8].

One of the key milestones of transplantology was marked by the discovery, in the middle of the last century, of immunosuppressive drugs – calcineurin inhibitors – which became the basis of therapy that prevents graft rejection response [9]. At the same time, the need for lifelong use of immunosuppressive drugs comes with a number of negative effects, among which the nephrotoxic effect, the so-called CNI nephrosclerosis, especially critical for kidney recipients, is the main one. The risk of developing cellular and humoral rejection persists

Table 3

Comparative analysis of laboratory parameters in recipients with and without graft dysfunction

Indicator	Normal function	Graft dysfunction	Significance level (p)
Creatinine, μmol/L	85.30 [71.50; 95.00]	250.05 [160.76; 425.23]	<0.00001
Urea, mmol/l	7.69 [6.20; 8.80]	19. 88 [12.86; 28.10]	<0.00001
Proteinuria, g/L	0.03 [0.03; 0.04]	0.14 [0.04; 0.40]	<0.00001
GFR, mL/min	81.30 [68.50; 100.00]	20.80 [11.35; 36.50]	<0.00001
TGF-β1, ng/mL	86.41 [69.48; 109.70]	111.40 [87.06; 145.15]	0.0004

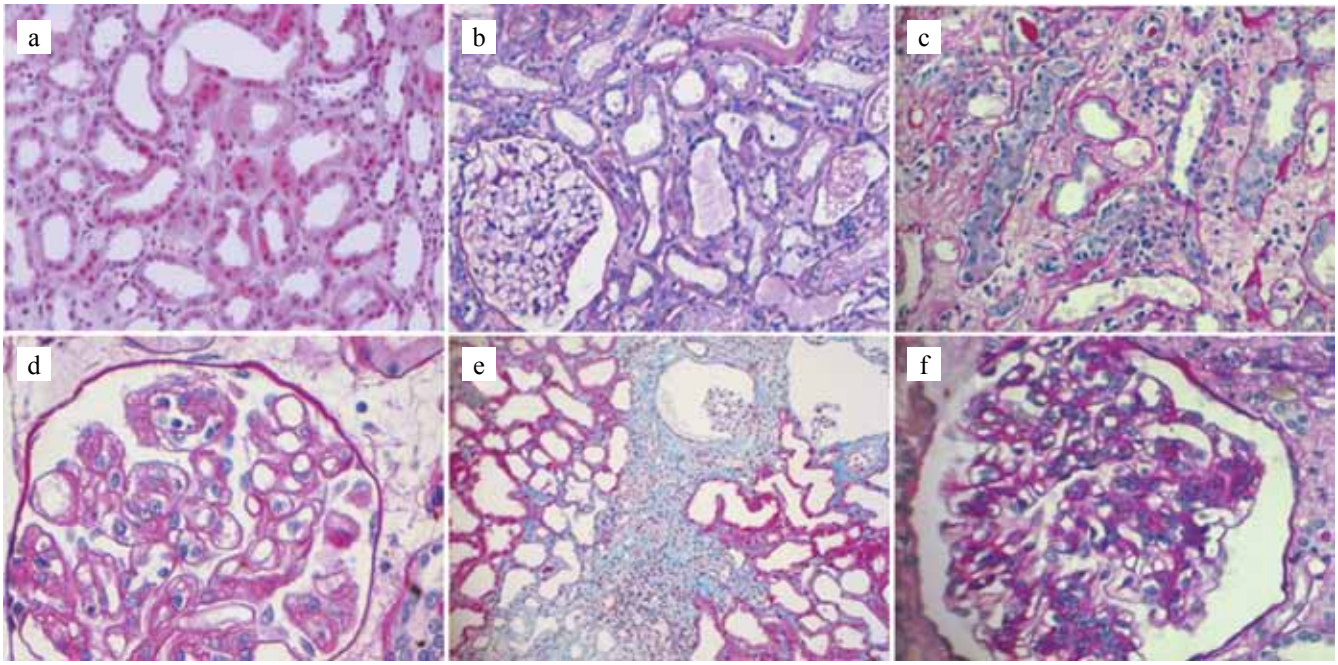

Fig. 1. Image of kidney biopsy specimens with H&E stain: (a) Normal, Masson's Trichrome stain ×40; (b) Acute tubular necrosis (ATN), PAS stain ×100; (c) Acute T-cell mediated rejection (ACR), PAS stain ×100; (d) Acute antibody-mediated rejection, transplant glomerulopathy (AMR), PAS stain ×200; (e) Interstitial fibrosis in CNI nephrotoxicity, Masson's Trichrome stain ×40; (f) Recurrent glomerulonephritis (IgA nephropathy)

throughout the life of the recipient. Repeated episodes of rejection lead to its chronic form with subsequent fibrosis and functional remodeling of the graft [10].

Kidney transplant injury is verified by morphological analysis of biopsy samples. However, the diagnostic value of this analysis is limited by the risk of taking an uninformative area of tissue, and the decision to perform an unscheduled biopsy is often made in the presence of a clear clinical picture of reduced renal function [11]. Studies on immune mechanisms of graft injury and improvement of diagnostic methods using minimally invasive laboratory technologies will allow not only to identify effective biomarkers of graft pathology at the early stage of complications, but also to consider them as a target for therapy.

Many studies have shown that TGF-β1 has a bright prospect as a marker for CKD [12]. Mediators of the TGF-β1 biological functions are Smad signaling pathways, including both Smad3, which is involved in the pathogenesis of kidney injury and fibrosis [13], and Smad2 and Smad7, which have a nephroprotective effect. These explain the ambiguity of data published by different authors on the role of TGF-β1 in kidney transplantation [14].

Considering our own and published data on the variability of the diagnostic and prognostic potential of TGF-β1 in solid organ recipients [7], this work was

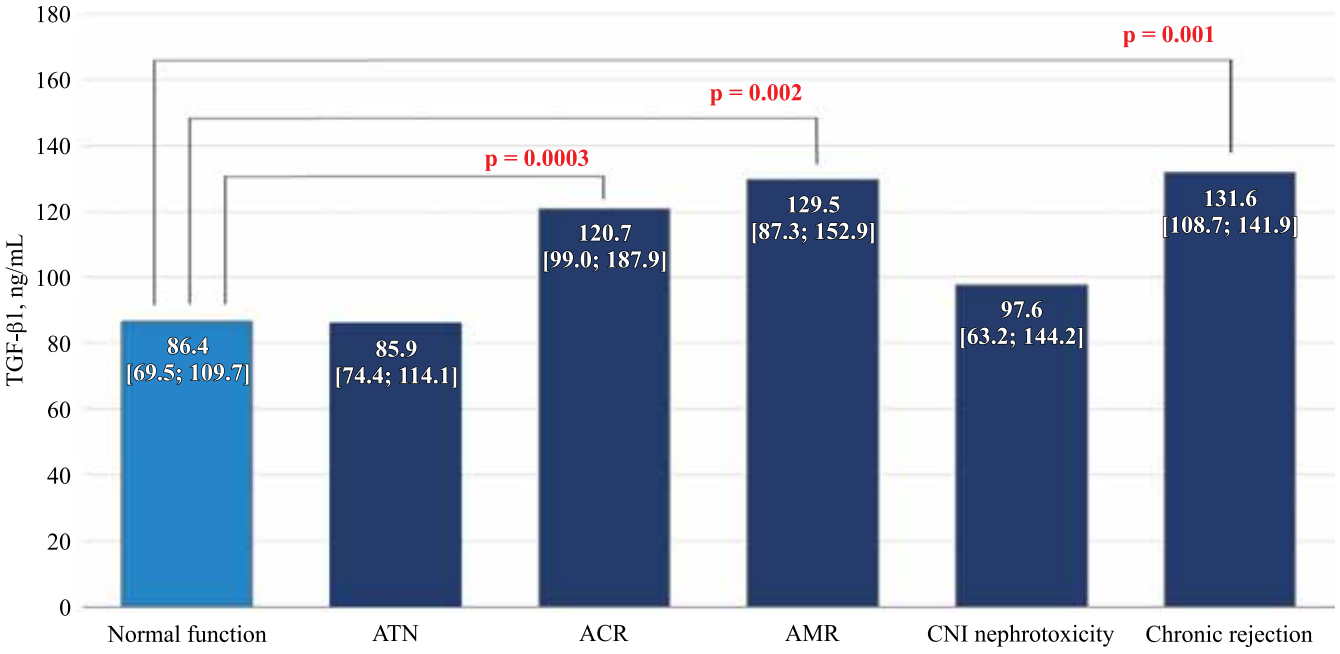

Fig. 2. Comparative analysis of serum TGF-β1 levels in kidney recipients with and without graft dysfunction of different nature

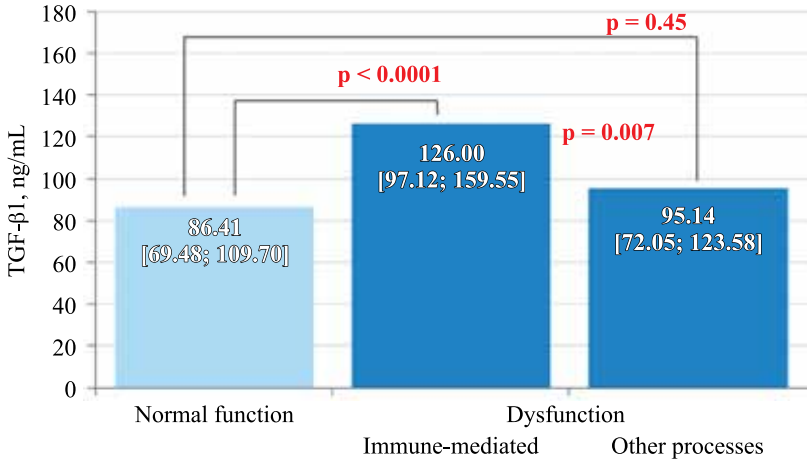

Fig. 3. Comparative analysis of TGF-β1 levels in kidney recipients with normal graft function, with immune-mediated graft dysfunction (acute cellular, humoral and chronic rejection), and with other processes (acute tubular necrosis, CNI nephrotoxicity)

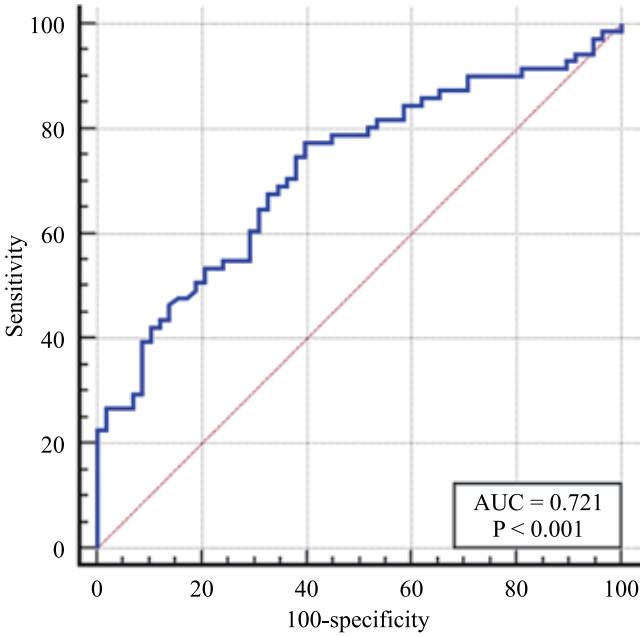

Fig. 4. ROC curve of serum TGF-β1 levels in kidney recipients with immune-mediated graft dysfunction

aimed at studying the diagnostic value of TGF-β1 in recipients with renal transplant dysfunction.

The study results showed that kidney recipients with serum TGF-β1 levels above 94.3 ng/mL had a 2.2-fold higher risk of acute or chronic rejection than other kidney recipients. In turn, differentiation of acute and chronic rejection requiring different approaches to therapy is possible by morphological examination.

Data on the association of high serum TGF-β1 levels with the presence of immune-mediated kidney graft injury are consistent with the results of foreign colleagues who showed higher TGF-β1 levels in kidney recipients with chronic rejection in comparison with recipients without rejection in the long term after transplantation [15]. However, it is worth noting that all patients had a history of several episodes of acute rejection, which allowed the authors to characterize TGF-β1 only as a marker for chronic rejection. In the present study, we have shown a significantly higher level of TGF-β1 in kidney recipients with acute rejection and in the early stages after transplantation, which allows us to count on the prospects of its use for identifying patients at risk of immune-mediated complications starting from the first days after KT.

Numerous animal experiments described by foreign authors demonstrate TGF-β1 participation in kidney injury mechanisms, as well as the association of increased TGF-β1 expression with decreased GFR, signs of tubular necrosis and fibrosis [16]. The positive correlation between TGF-β1 level and proteinuria ($r = 0.280$; $p = 0.001$), which we found, seems to be very significant taking into account the results of experiments by Kasuga et al., who managed to reduce the level of proteinuria in rats with glomerulonephritis by administering TGFβRII receptor antibodies [17]. This suggests that TGF-β1 can be used as a target for therapy. In another study by Du X.X. et al., serum TGF-β1 level was found to correlate with GFR and nephrograft survival period [18], which indicates a probable influence of a number of associated factors on TGF-β1 level that require additional study.

A study by Sugimoto et al. showed that in mice, morphogenic protein BMP, a member of the TGF-β superfamily, acts as an antagonist of TGF-β signal transduction, and oral administration of its agonist (THR-123) inhibits renal fibrosis [19]. Another activator of TGF-β signaling is thrombospondin-1, whose inhibition in mice, according to Sun et al., resulted in activation of angiogenesis and reduction of renal fibrosis in mice [20]. In a model of KT in rats, administration of the anti-inflammatory drug pirfenidone, which targets TGF-β, resulted in attenuation of inflammation and renal fibrosis [21].

In an experimental work by Border et al., an attempt was made to inhibit the process of fibrosis in glomerulonephritis by administering antibodies to TGF-β1 [22]. The experiments resulted in effective suppression of extracellular matrix protein accumulation, which was confirmed by histological studies [23]. Therapeutic use of TGF-β1 inhibitors is not yet possible due to the ambiguous role of the latter in tissue homeostasis and regeneration, which manifests both pro- and antifibrotic effects. It is suggested that the combination of antifibrotic therapy with protection of the tubular epithelium may be very promising [24].

The development of acute and chronic rejection based on immune mechanisms, contributes to accelerated formation of graft fibrosis [25]. Early detection of graft dysfunction is crucial for renoprotective treatment and can positively influence transplant outcomes.

Predicting allograft survival remains challenging, but a combination of clinical data and studies of potential biomarkers of the pathology can improve diagnostic accuracy. To date, several potential antifibrotic strategies have been identified, but no specific drug has yet been approved for the treatment of kidney transplant recipients because of the complexity of the cascade of pathologic processes in fibrosis and the intersection of many signaling pathways that mutually influence and compensate each other.

There is a need to develop ancillary minimally invasive diagnostic technologies, biomarkers to predict long-term outcome of transplantation or to differentiate fibrosis resulting from causes of different nature [26].

In the present study, a threshold serum TGF-β1 level in kidney recipients was calculated to identify patients at high risk of acute or chronic rejection who were recommended for unscheduled biopsy.

Obviously, all peculiarities of the mechanisms of TGF-β1 involvement in the development of transplanted kidney pathology are subject to further in-depth study.

The authors declare no conflict of interest.

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