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BIOMARKERS OF RENAL TRANSPLANT FIBROSIS

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Fibrosis is one of the causes of kidney allograft loss, especially late after transplantation (up to 65% incidence after 2 years). The purpose of this literature review is to analyze studies examining noninvasive monitoring techniques for renal graft fibrosis.

Keywords: fibrosis, kidney transplantation, biomarkers.

Renal allograft fibrosis is a complex, dynamic and inevitable process that is the terminal stage of most progressive kidney transplant diseases. A number of studies have demonstrated that the progression of interstitial fibrosis is particularly noticeable in the first hours after transplantation (which may be a window for therapeutic intervention) and can be detected in kidney recipients even with good graft function [1]. Fibrosis can affect all parts of the kidney, namely the tubulointerstitium, the glomeruli (glomerulosclerosis) and the vessels (atherosclerosis and arteriolosclerosis).

In renal allografts, interstitial fibrosis and tubular atrophy are evaluated together because the two phenomena almost inevitably occur in parallel [2, 3]. Interstitial fibrosis and tubular atrophy (IF/TA) (hereafter referred to as renal allograft fibrosis) is detected in approximately 40% of renal allografts after 3–6 months and increases to approximately 65% of cases 2 years after transplantation; characterized by profound renal tissue remodeling, excessive formation/deposition of extracellular matrix fibrillar cells, which leads to impaired tissue architecture and microperfusion, which in turn reduces renal graft function [4]. In patients who return to dialysis therapy or require retransplantation, the most common cause of decreased allograft function is IF/TA, regardless of the primary cause of the transplanted kidney fibrosis. The degree of fibrosis affects kidney graft function and survival [5].

The clinical impact of IF/TA was first described in 2009 [6]. Several studies have highlighted the negative impact of this condition on major clinical outcomes, and it has also been suggested that IF/TA may be associated with inadequate immunosuppressive therapy and usually precedes chronic active T cell-mediated rejection [7].

New molecular and pathogenetic insights into the biological mechanisms associated with kidney graft fibrosis provide an opportunity to identify new potential biomarkers and select new, clinically valuable therapeutic targets, which is a major goal of research in nephrology and organ transplantation.

MECHANISM OF FIBROSIS

Native renal fibrosis and IF/TA in renal allografts probably have common mechanisms and pathophysiology of the process. However, development of fibrosis in the renal allograft is a multifactorial process and may be a consequence of pre-existing pathology of the donor organ, acute cellular, antibody-mediated (humoral) or mixed rejection crises, diabetes, ischemic and hypertensive damage to the graft, chronic nephrotoxicity, cytomegalovirus infection, and the number of biopsies performed on the renal transplant [3].

At the initial stage of the profibrotic process inside the graft, inflammation is initiated, which is an integral part of the body's defense mechanisms in response to damage. This phenomenon in the early stage of renal fibrosis is potentially reversible. However, if the fibrosis progresses, the extracellular matrix proteins undergo several biochemical modifications that make it irreversible [8].

The renin-angiotensin-aldosterone system (RAAS), hypoxia, acute cellular rejection and chronic inflammation, etc., are involved in the pathogenesis of IF/TA. Some of these pathways are partially induced by immunosuppressive therapy [9–12].

Tubular and glomerular cells produce proinflammatory cytokines depending on the etiology of kidney damage. In addition, inflammatory infiltrates (including neutrophils, macrophages, T cells and B cells) enhance the fibrotic process and, by activating endothelial cells of peritubular capillaries, can promote attraction of new interstitial mononuclear cells. Following neutrophils, macrophages infiltrate the damaged tissue, phagocytize and secrete fibrotic cytokines, leading to proliferation of fibroblasts and myofibroblasts, epithelial-mesenchymal

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transition (EMT) [5, 13], excessive accumulation of extracellular matrix (ECM) and pathological proteins not normally identified in renal tissue [3, 14]. Macrophages are the main source of transforming growth factor beta 1 (TGF-β1), a powerful chemoattractant for monocytes and macrophages, which play a major role in renal allograft fibrosis [15].

As reported by Toki et al. in protocol renal allograft biopsies one year after transplantation, macrophage infiltration at 1 year correlated with renal dysfunction at 1, 12 and 36 months posttransplant [16]. It is interesting to suggest that the assessment of macrophage infiltration at early renal transplant biopsy may have value for the subsequent prognosis of transplanted kidney function.

There are still debates about additional myofibroblast progenitor cells, including circulating cells originating from bone marrow, or about the transition from macrophages, epithelial or endothelial cells [17, 18]. In the kidney, EMT describes the transition and cellular migration of polarized epithelial tubule cells across the basal membrane to apolar mesenchymal cells in the interstitium. Mesenchymal cells can actively secrete components of extracellular matrix – collagens, fibronectin – which can contribute to scar formation [14, 19]. Evidence for EMT is convincing in studies conducted in vitro, but there is no such evidence in in vivo studies. Studies in rats have shown that EMT is involved in the development of IF/TA, and it correlates with increased oxidative stress. A correlation between EMT 3 months after kidney transplantation and late graft lesions expressed in IF/TA, observed 1 year after transplantation, has also been reported [9, 20, 21].

Other potential extracellular matrix-producing cells are fibrocytes, a multitude of circulating bone marrow monocytes with fibroblast-like properties, which, in the presence of profibrotic cytokines such as IL-4 and IL-13, differentiate and infiltrate the renal parenchyma and participate in fibrogenesis [8].

The term "oxidative stress" refers to the damage caused by the accumulation of reactive oxygen species in cells and tissues [22, 23]. During this condition, cells undergo profound functional and morphological changes: hyperexpression of mesenchymal markers (vimentin, smooth muscle alpha-actin, fibronectin), release of matrix metallopeptidase (MMP)-9 and -2, increased motility, decreased cytokeratin and E-cadherin levels and changes in heparan sulfate proteoglycans (HSPGs) [24, 25]. The most abundant HSPGs on renal tubular epithelial cells is syndecan-1, a factor that promotes renal tubule survival and repair after damage, and its level correlates with improved function of the injured kidney allograft. This factor appears to be regulated by several factors, including heparanase, endo-β-D-glucuronidase, which are involved in the pathogenesis of several renal diseases, especially in diabetic nephropathy, and are suggested to be involved in allograft pathology [26].

Kidney graft toxicity associated with immunosuppressive drugs, particularly calcineurin inhibitors, can provoke oxidative stress by disassociating the mitochondrial system of oxidative phosphorylation mediated by Ca^{++} increase [27, 28, 29]. Fibrotic changes that are secondary to these events can cause chronic graft hypoxia with activation of various biochemical mediators, including hypoxia-inducible factor, which activates a large number of target genes involved in the maintenance of homeostasis during hypoxia, such as vascular endothelial growth factor (VEGF), erythropoietin, epidermal growth factor receptor (EGFR) and platelet-derived growth factor (PDGF) [30].

All these events are accompanied by significant morphological changes (including architectural changes in the renal tubules, apoptosis, defects in cell cycle progression, microvascular rarefaction) leading to tubular atrophy, a condition that has ever been associated with allograft fibrosis [31, 32].

DIAGNOSIS OF RENAL ALLOGRAFT FIBROSIS

Instrumental methods. In renal allografts, ultrasonography and magnetic resonance imaging (MRI) are the two main instrumental methods for assessing fibrosis. There have been suggestions that ultrasound elastography for tissue elasticity estimation, an approach that has been relatively well established in assessing liver fibrosis, correlates with fibrosis in renal allografts, but several investigators have found no such correlation [33].

MRI-based elastography is an alternative approach, but the first results of a study of renal allografts with fibrosis by this method showed that renal tissue stiffness changes much less in fibrosis than in the liver, suggesting that elastography of transplanted kidneys may not be sensitive enough to assess fibrosis [34]. In the liver, tissue stiffness increases significantly with increasing fibrosis, whereas data on the stiffness and biomechanical properties of the kidneys at different degrees of fibrosis are lacking.

Pulsed-wave Doppler, in which a quantitative assessment of blood flow (absolute parameters: maximum systolic blood flow velocity and final minimum diastolic velocity; relative parameters: resistance index and pulse index) in vessels on the curve that is reflecting Doppler frequency shift spectrum, performed in the post-transplant period, is of great importance for prediction of renal transplant outcomes [35, 36]. In a study by Pykov M.I., it was shown that as IF/TA progressed, kidney graft function decreased, which was manifested in increased proteinuria, serum creatinine levels and decreased glomerular filtration rate ($p < 0.001$). At the same time, the more pronounced the fibrotic changes, the lower the peak systolic and end-diastolic velocities, resistance index and pulsatility index [37].

Morphological analysis methods. To date, the most accurate method of imaging and diagnosis of kidney graft pathology is punch biopsy. Even when clinical evaluation conclusively indicates the specific cause of allograft dysfunction, biopsy is still necessary to clarify the degree and severity of renal tissue damage and choose the most optimal treatment tactics [3, 20, 38]. In addition to biopsy "by indication", some centers perform biopsy "by protocol" to detect subclinical chronic conditions and track the progression of renal fibrosis, in particular, its quantitative assessment [5].

Servais et al. demonstrated that kidney transplant biopsies obtained at day 0, month 3 and month 12 showed a rapid progression of IF/TA from 19% to 27% at month 3 and 32% at month 12 after kidney transplantation [39]. Serum creatinine levels and glomerular filtration rate (GFR) played a limited clinical role in assessing histopathological changes in the graft.

A kidney biopsy is an invasive method of diagnosing graft pathology, and the procedure also requires hospitalization. Like any invasive procedure, kidney biopsy also has a number of complications; therefore, noninvasive, sensitive and etiologically specific biomarkers for the diagnosis of pathological processes in a transplanted kidney are essential [40].

BIOMARKERS OF TRANSPLANTED KIDNEY FIBROSIS

The ideal biomarker should be noninvasive, reflect the degree and dynamics of renal fibrosis treatment, and be more sensitive than established other diagnostic and imaging techniques [41, 42]. It is important to note that at present, none of the identified markers is specific for transplanted kidney fibrosis, but rather may reflect other processes occurring in the body [43].

Transforming growth factor beta (TGF-β) is a cytokine involved in the initiation of various cellular processes (regulation of cell proliferation, apoptosis, cell migration and differentiation, leads to the synthesis of extracellular matrix proteins by myofibroblasts) and is the main mediator of renal fibrosis due to the EMT signaling pathway activation [1, 30, 44]. One of the three main isoforms, TGF-β1, has the greatest biological and pathological effect [45].

A review of the literature on the pathogenetic significance of TGF-β in the development of renal fibrosis showed that TGF-β hyperactivation via signaling pathways occurs in renal tissue damage of various origins [23, 45, 46]. It is likely that TGF-β expression may have prognostic significance in assessing kidney transplant survival [47]. The TGF-β gene has a significant polymorphism, which presumably may be responsible for the genetically determined cytokine activity and its association with various diseases. A high-producing TGF-β1 genotype in combination with other cytokines is a risk factor for chronic graft nephropathy [48, 49].

It has been experimentally shown that anti-TGF-β therapy in rats reduces chronic rejection [50], and mycophenolic acid can inhibit allograft fibrosis by suppressing TGF-β effects [51]. TGF-β inhibition is not without potential serious side effects: firstly, TGF-β is a tumor suppressor, and its inhibition can accelerate tumor progression [52]. In vivo modulation of cyclosporine effects by altering TGF-β levels has been demonstrated to partially mediate the beneficial and undesirable effects of cyclosporine [53].

Galectin-3. The mechanism of action of galectin-3 (a family of beta-galactoside-binding proteins) may vary depending on its localization: inside the cell, it helps protect cells from apoptosis; outside the cell, its action, on the contrary, promotes cell death [54]. It has been established that at the site of damage, galectin-3 is secreted into the extracellular space, stimulating the process of fibrosis through activation and proliferation of resting fibroblast cells. There are new studies of the association of galectin-3 with kidney graft dysfunction in the long term after transplantation [55, 56]. Based on a retrospective analysis, it was shown that serum galectin-3 levels were elevated in kidney transplant recipients, and independently associated with increased risk of late graft failure; the results were independent of donor, recipient, and graft characteristics, including GFR [21]. Further studies are warranted to evaluate whether galectin-3-targeted therapy may represent a novel opportunity to decrease the high burden of late graft failure. Recipients with high galectin-3 levels, high systolic blood pressure (≥140 mmHg), and/or a history of smoking are at particularly high risk of kidney graft failure [21].

Platelet-derived growth factor (PDGF). In the PDGF family, three isoforms PDGF-B, -C and -D, as well as both receptors (a and b) are involved in the mechanisms of renal fibrosis [57, 58]. A study by E.M. Buhl et al. shows physiological PDGFR-β signaling in renal mesenchymal cells as important for normal renal development. PDGFR-β activation was sufficient to trigger progressive renal fibrosis, and this created a unique model to specifically study the effects, reversibility, and therapeutic interventions in renal fibrosis independent of inflammation, hypertension, or epithelial or endothelial damage [59].

Vascular endothelial growth factors (VEGF) are powerful angiogenic factors produced by macrophages, fibroblasts, hepatocytes, endothelial and other cells [60]. They participate in activation, proliferation, migration and differentiation of blood and lymphatic vessel endothelial cells by interacting with them through specific tyrosine kinase receptors [61].

Inflammation plays a crucial role in the initiation and development of renal fibrosis. Signal transduction via VEGF-C, VEGF-D, and VEGF receptor (VEGFR)-3 is a central molecular mechanism of lymphangiogenesis.

TGF-β induces peritoneal fibrosis in association with peritoneal dialysis and also induces peritoneal neoangiogenesis through interaction with VEGF-A. On the other hand, TGF-β has a direct inhibitory effect on the growth of lymphatic endothelial cells. Hiroshi Kinashi proposed a possible mechanism of the TGF-β/VEGF-C pathway in which TGF-β promotes VEGF-C production in tubular epithelial cells, macrophages, and mesothelial cells, leading to lymphangiogenesis in renal and peritoneal fibrosis. Connective tissue growth factor (CTGF) is also involved in fibrosis-associated renal lymphangiogenesis through interaction with VEGF-C, in part by mediating TGF-β signaling. Further clarification of the mechanism might lead to the development of new therapeutic strategies to treat fibrotic diseases [62].

Ying Zhang and colleagues suggested that there is a close relationship between macrophages and lymphatic endothelial progenitor cells in renal fibrosis. The study demonstrated that lymphangiogenesis was positively correlated with the degree of fibrosis and macrophage infiltration. Compared to resting (M0) macrophages and alternatively activated (M2) macrophages, classically activated (M1) macrophages predominantly transdifferentiated into lymphatic endothelial cells (LECs) in vivo and in vitro. VEGF-C further enhanced polarization and transdifferentiation of M1 macrophages into LECs by activating VEGFR3. It was suggested that VEGF-C/VEGFR3 pathway activation downregulates macrophage autophagy and subsequently regulates the macrophage phenotype. The induction of autophagy in macrophages by rapamycin decreased M1 macrophage polarization and differentiation into LECs. These results suggest that M1 macrophages promote lymphangiogenesis and contribute to newly formed lymphatic vessels in the renal fibrosis microenvironment [63].

MicroRNAs (miRNAs). A separate group of signaling molecules considered as promising candidates for the role of biomarkers of post-transplant complications in kidney transplant recipients are miRNAs, small noncoding RNAs (18 to 25 nucleotides) that regulate gene expression and play an important role in regulating the functions of both healthy and damaged cells [64, 65]. Currently, very few studies on the role and diagnostic significance of miRNAs in post-transplant complications in kidney recipients have been published. At the same time, new data on the functions of currently known microRNA molecules appear, for example, miR-144 demonstrates the involvement in the cascade of processes forming the syndrome of obliterating bronchiolitis in lung recipients [66]; increased miR-155 expression is associated with lung and kidney graft dysfunction [67, 68]; an association of miR-21, -122 levels in solid organ recipients with long-term graft outcomes has been shown [69].

Signaling molecules miR-21 [70], miR-214 [71] and miR-192 [72] have been shown to be profibrotic, whereas the miR-29 family [73], miR-200b [74] and miR-30e [75] are antifibrotic. It has been suggested that most miRs target TGF-β signaling to collagen expression or metabolic pathways. The TGF-β/Smad3 pathways play an important role in fibrosis. When nephrons are damaged, TGF-β signaling is activated, thereby stimulating the TGF-β1 receptor, which then activates the Smad3 pathway. In the context of renal fibrosis, Smad3 is pathogenic, whereas Smad7 is protective. MiR-433 is an important component of the TGF-β/Smad3 pathway, creates a positive feedback loop, and enhances TGF-β/Smad3 signaling. In vitro and in vivo expression of miR-433 regulates the development of fibrosis, which in turn is induced by TGF-β1, by enhancing the antizyme inhibitor Azin1, an important regulator of polyamine synthesis [76]. Chung et al. reported that miR-192 mediates TGF-β/Smad3-regulated renal fibrosis [72]. Further study of the biological functions of microRNAs and their expression profile is required for possible use in clinical practice as a potential predictor of complications.

CONCLUSION

The search for a non-invasive method of detecting fibrosis before the development of irreversible complications in a transplanted kidney is an important task in transplantology. Three potential biomarkers involved in the development of kidney transplant pathology – TGF-β, galectin-3 and microRNA – can be highlighted in the development of noninvasive diagnostic methods for allograft kidney fibrosis. They present new diagnostic opportunities and open up new therapeutic targets.

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REFERENCES

1. *Nankivell BJ, Borrows RJ, Fung CL, O'Connell PJ, Chapman JR, Allen RD*. Delta analysis of posttransplantation tubulointerstitial damage. *Transplantation*. 2004; 78 (3): 434–441.
2. *Stolyarevich ES, Tomilina NA*. Understanding evolution on the causes of late renal allograft dysfunction. *Russian Journal of Transplantology and Artificial Organs*. 2015; 17 (2): 113–115.
3. *Granata S, Benedetti C, Gambaro G, Zaza G*. Kidney allograft fibrosis: what we learned from latest translational research studies. *Journal of Nephrology*. 2020; 33 (6): 1201–1211.
4. *Boor P, Floege J.* Renal allograft fibrosis: Biology and therapeutic targets. *American Journal of Transplantation*. 2015; 15 (4): 863–886.
5. *Saritas T, Kramann R.* Kidney Allograft Fibrosis: Diagnostic and Therapeutic Strategies. *Transplantation*. 2021; 105 (10): e114–e130.
6. *Mannon RB, Matas AJ, Grande J, Leduc R, Connett J, Kasiske B et al.* Inflammation in areas of tubular atrophy in kidney allograft biopsies: a potent predictor of allograft failure. *Am J Transplant*. 2010; 10 (9): 2066–2073.

7. *Modena BD, Kurian SM, Gaber LW, Waalen J, Su AI, Gelbart T et al.* Gene Expression in Biopsies of Acute Rejection and Interstitial Fibrosis/Tubular Atrophy Reveals Highly Shared Mechanisms That Correlate With Worse Long-Term Outcomes. *Am J Transplant*. 2016; 16 (7): 1982–1998.
8. *Shao DD, Suresh R, Vakil V, Gomer RH, Pilling D*. Pivotal Advance: Th-1 cytokines inhibit, and Th-2 cytokines promote fibrocyte differentiation. *J Leukoc Biol*. 2008; 83 (6): 1323–1333.
9. *Bontha SV, Maluf DG, Archer KJ, Dumur CI, Dozmorov MG, King AL et al*. Effects of DNA Methylation on Progression to Interstitial Fibrosis and Tubular Atrophy in Renal Allograft Biopsies: A Multi-Omics Approach. *Am J Transplant*. 2017; 17 (12): 3060–3075.
10. *Lipphardt M, Song JW, Matsumoto K, Dadafarin S, Dihazi H, Müller G, Goligorsky MS*. The third path of tubulointerstitial fibrosis: aberrant endothelial secretome. *Kidney Int*. 2017; 92 (3): 558–568.
11. *Melk A, Schmidt BM, Vongwiwatana A, Rayner DC, Halloran PF*. Increased expression of senescence-associated cell cycle inhibitor p16INK4a in deteriorating renal transplants and diseased native kidney. *Am J Transplant*. 2005; 5 (6): 1375–1382.
12. *Rosenberger C, Eckardt KU.* Oxygenation of the Transplanted Kidney. *Semin Nephrol*. 2019; 39 (6): 554–566.
13. *Land W, Schneeberger H, Schleibner S, Illner WD, Abendroth D, Rutili G et al*. The beneficial effect of human recombinant superoxide dismutase on acute and chronic rejection events in recipients of cadaveric renal transplants. *Transplantation*. 1994; 57 (2): 211–217.
14. *Sun YB, Qu X, Caruana G, Li J*. The origin of renal fibroblasts/myofibroblasts and the signals that trigger fibrosis. *Differentiation*. 2016; 92 (3): 102–107.
15. *Nikolic-Paterson DJ, Wang S, Lan HY.* Macrophages promote renal fibrosis through direct and indirect mechanisms. *Kidney Int Suppl*. 2014; 4 (1): 34–38.
16. *Toki D, Zhang W, Hor KL, Liuwantara D, Alexander SI, Yi Z et al*. The role of macrophages in the development of human renal allograft fibrosis in the first year after transplantation. *Am J Transplant*. 2014; 14 (9): 2126–2136.
17. *Armulik A, Abramsson A, Betsholtz C.* Endothelial/pericyte interactions. *Circ Res*. 2005; 97 (6): 512–523.
18. *Goodall KJ, Poon IK, Phipps S, Hulett MD*. Soluble heparan sulfate fragments generated by heparanase trigger the release of pro-inflammatory cytokines through TLR-4. *PLoS One*. 2014; 9 (10): e109596.
19. *Kramann R, Schneider RK, DiRocco DP, Machado F, Fleig S, Bondzie PA et al*. Perivascular Gli1+ progenitors are key contributors to injury-induced organ fibrosis. *Cell Stem Cell*. 2015; 16 (1): 51–66.
20. *Quaglia M, Merlotti G, Guglielmetti G, Castellano G, Cantaluppi V*. Recent Advances on Biomarkers of Early and Late Kidney Graft Dysfunction. *Int J Mol Sci*. 2020; 21 (15): 5404.
21. *Sotomayor CG, Te Velde-Keyzer CA, Diepstra A, van Londen M, Pol RA, Post A et al.* Galectin-3 and Risk of Late Graft Failure in Kidney Transplant Recipients: A 10-year Prospective Cohort Study. *Transplantation*. 2021; 105 (5): 1106–1115.
22. *Djamali A*. Oxidative stress as a common pathway to chronic tubulointerstitial injury in kidney allografts. *Am J Physiol Renal Physiol*. 2007; 293 (2): F445–455.
23. *Göttmann U, Oltersdorf J, Schaub M, Knoll T, Back WE, van der Woude FJ et al.* Oxidative stress in chronic renal allograft nephropathy in rats: effects of long-term treatment with carvedilol, BM 91.0228, or alpha-tocopherol. *J Cardiovasc Pharmacol*. 2003; 42 (3): 442–450.
24. *Celie JW, Rutjes NW, Keuning ED, Soininen R, Heljasvaara R, Pihlajaniemi T et al.* Subendothelial heparan sulfate proteoglycans become major L-selectin and monocyte chemoattractant protein-1 ligands upon renal ischemia/reperfusion. *Am J Pathol*. 2007; 170 (6): 1865–1878.
25. *Carew RM, Wang B, Kantharidis P.* The role of EMT in renal fibrosis. *Cell Tissue Res*. 2012; 347 (1): 103–116.
26. *Garsen M, Rops ALWMM, Rabelink TJ, Berden JHM, van der Vlag J.* The role of heparanase and the endothelial glycocalyx in the development of proteinuria. *Nephrol Dial Transplant*. 2014; 29 (1): 49–55.
27. *Nieuwenhuijs-Moeke GJ, Pischke SE, Berger SP, Sanders JSF, Pol RA, Struys MMRF et al*. Ischemia and Reperfusion Injury in Kidney Transplantation: Relevant Mechanisms in Injury and Repair. *J Clin Med*. 2020; 9 (1): 253.
28. *Salvadori M, Rosso G, Bertoni E*. Update on ischemia-reperfusion injury in kidney transplantation: Pathogenesis and treatment. *World J Transplant*. 2015; 5 (2): 52.
29. *Bedi S, Vidyasagar A, Djamali A*. Epithelial-to-mesenchymal transition and chronic allograft tubulointerstitial fibrosis. *Transplant Rev (Orlando)*. 2008; 22 (1): 1–5.
30. *Richter K, Kietzmann T.* Reactive oxygen species and fibrosis: further evidence of a significant liaison. *Cell Tissue Res*. 2016; 365 (3): 591–605.
31. *Liu Y.* Cellular and molecular mechanisms of renal fibrosis. *Nat Rev Nephrol*. 2011; 7 (12): 684–696.
32. *Yang L, Besschetnova TY, Brooks CR, Shah JV, Bonventre JV*. Epithelial cell cycle arrest in G2/M mediates kidney fibrosis after injury. *Nat Med*. 2010; 16 (5): 535–543.
33. *Desvignes C, Dabadie A, Aschero A, Ruocco A, Garaix F, Daniel L et al*. Technical feasibility and correlations between shear-wave elastography and histology in kidney fibrosis in children. *Pediatr Radiol*. 2021; 51 (10): 1879–1888.
34. *Ma MK, Law HK, Tse KS, Chan KW, Chan GC, Yap DY et al*. Non-invasive assessment of kidney allograft fibrosis with shear wave elastography: A radiological-pathological correlation analysis. *Int J Urol*. 2018; 25 (5): 450–455.
35. *McArthur C, Geddes CC, Baxter GM*. Early measurement of pulsatility and resistive indexes: correlation with long-term renal transplant function. *Radiology*. 2011; 259 (1): 278–285.
36. *Poleshchuk LA.* Kharakteristika pochechnoy gemodinamiki u detey s zabolevaniyami pochek (obzor literatury). *Nefrologiya i dializ*. 2006; 8 (3): 225–231.
37. *Pykov MI, Ektov DB, Vasil'yev KG, Kushnir BL, Martynenkova AV*. Parametry gemodinamiki pochechnogo transplantata s raznoy stepen'yu interstitsial'nogo fibroza i tubulyarnoy atrofii v otdalennom posttransplantatsi-

onnom periode u detey. *Vestnik Rossiyskogo nauchnogo tsentra rentgenoradiologii*. 2021; 21 (4): 138–154.
38. *Vanhove T, Goldschmeding R, Kuypers D.* Kidney fibrosis: origins and interventions. *Transplantation*. 2017; 101 (4): 713–726.
39. *Servais A, Meas-Yedid V, Noël LH, Martinez F, Panterne C, Kreis H et al.* Interstitial fibrosis evolution on early sequential screening renal allograft biopsies using quantitative image analysis. *Am J Transplant*. 2011; 11 (7): 1456–1463.
40. *Vahed SZ, Samadi N, Ardalan M*. Transplantation diagnosis of interstitial fibrosis and tubular atrophy in kidney allograft implementation of MicroRNAs. *Iranian Journal of Kidney Diseases*. 2014; 8 (1): 4–12.
41. *Genovese F, Manresa AA, Leeming DJ, Karsdal MA, Boor P.* The extracellular matrix in the kidney: a source of novel non-invasive biomarkers of kidney fibrosis? *Fibrogenesis Tissue Repair*. 2014; 7 (1): 1–4.
42. *Hartono C, Muthukumar T, Suthanthiran M.* Noninvasive diagnosis of acute rejection of renal allografts. *Current Opinion in Organ Transplantation*. 2010; 15: 35–41.
43. *Saritas T, Kramann R.* Kidney allograft fibrosis: diagnostic and therapeutic strategies. *Transplantation*. 2021; 105 (10): e114–e130.
44. *Nankivell BJ, P'Ng ChH, O'Connell PhJ, Chapman JR.* Calcineurin inhibitor nephrotoxicity through the lens of longitudinal histology: comparison of cyclosporine and tacrolimus eras. *Transplantation*. 2016; 100 (8): 1723–1731.
45. *Manfro RC, Aquino-Dias EC, Joelsons G, Nogare AL, Carpio VN, Gonçalves LF.* Noninvasive Tim-3 messenger RNA evaluation in renal transplant recipients with graft dysfunction. *Transplantation*. 2008; 86 (12): 1869–1874.
46. *Isaka Y.* Targeting TGF-β Signaling in Kidney Fibrosis. *Int J Mol Sci.* 2018; 19 (9): 2532. doi: 10.3390/ijms19092532.
47. *Nikolova PN, Ivanova MI, Mihailova S, Mihaylova A, Baltadjieva D, Simeonov PL et al.* Cytokine gene polymorphism in kidney transplantation – Impact of TGF-β1, TNF-α and IL-6 on graft outcome. *Transplant immunology*. 2008; 18 (4): 344–348.
48. *Mu HJ, Xie P, Chen JY, Gao F, Zou J, Zhang J, Zhang B.* Association of TNF-α, TGF-β1, IL-10, IL-6, and IFN-γ gene polymorphism with acute rejection and infection in lung transplant recipients. *Clin Transplant*. 2014; 28 (9): 1016–1024.
49. *Kurabekova RM, Gichkun OE, Meshcheryakov SV, Shevchenko OP*. Rol' polimorfizma gena transformiruyushchego faktora rosta β1 v razvitii oslozhneniy posle transplantatsii solidnykh organov. *Vestnik transplantologii i iskusstvennykh organov*. 2021; 23 (3): 180–185.
50. *Guan Q, Li S, Gao S, Chen H, Nguan CY, Du C.* Reduction of chronic rejection of renal allografts by anti-transforming growth factor-β antibody therapy in a rat model. *Am J Physiol Renal Physiol*. 2013; 305 (2): F199–207.
51. *Djamali A, Vidyasagar A, Yagci G, Huang LJ, Reese S.* Mycophenolic acid may delay allograft fibrosis by inhibiting transforming growth factor-beta1-induced activation of Nox-2 through the nuclear factor-kappaB pathway. *Transplantation*. 2010; 90 (4): 387–393.
52. *Garber K*. Companieswaver in efforts to target transforming growth factor beta in cancer. *Journal of the National Cancer Institute*. 2009; 101: 1664–1667.
53. *Khanna AK, Cairns VR, Becker CG, Hosenpud JD*. Transforming growth factor (TGF)-beta mimics and anti-TGF-beta antibody abrogates the *in vivo* effects of cyclosporine: Demonstration of a direct role of TGF-beta in immunosuppression and nephrotoxicity of cyclosporine. *Transplantation*. 1999; 67: 882–889.
54. *Shevchenko OP, Ulybysheva AA, Gichkun OE, Mozheyko NP, Stakhanova EA, Kvan VS i dr.* Galektin-3 pri ottorzhenii i fibroze transplantirovannogo serdtsa. *Vestnik transplantologii i iskusstvennykh organov*. 2019; 21 (3): 145–150.
55. *Chen SC, Kuo PL*. The role of galectin-3 in the kidneys. *International Journal of Molecular Sciences*. 2016; 17 (4): 565.
56. *Gyamdzhyan KA, Kukes VG, Maksimov ML.* Clinical value of determining galectin-3 in patients with chronic heart failure. *Medical Council. Remedium*. 2017; 7: 63–68.
57. *Ostendorf T, Eitner F, Floege J.* The PDGF family in renal fibrosis. *Journal of Pediatric Nephrology*. 2012; 27: 1041–1050.
58. *Boor P, Ostendorf T, Floege J.* PDGF and the progression of renal disease. *Nephrology Dialysis Transplantation*. 2014; 29 (Suppl 1): I45–I54.
59. *Ortiz A.* PDGFR-β and kidney fibrosis. *EMBO Mol Med*. 2020; 12 (3): e11729. doi: 10.15252/emmm.201911729.
60. *Kiseleva YeP, Krylov AV, Starikova EA, Kuznetsova SA*. Faktor rosta sosudistogo endoteliya i immunnaya sistema. *Uspekhi sovremennoy biologii*. 2009; 129 (4): 1–12.
61. *Taimeh Z, Loughran J, Birks EJ, Bolli R.* Vascular endothelial growth factor in heart failure. *Nature Reviews Cardiology*. 2013; 10: 519–530.
62. *Kinashi H, Ito Y, Sun T, Katsuno T, Takei Y.* Roles of the TGF-β–VEGF-C Pathway in Fibrosis-Related Lymphangiogenesis. *Int J Mol Sci*. 2018; 19 (9): 2487. doi: 10.3390/ijms19092487.
63. *Zhang Y, Zhang C, Li L, Liang X, Cheng P, Li Q et al.* Lymphangiogenesis in renal fibrosis arises from macrophages via VEGF-C/VEGFR3-dependent autophagy and polarization. *Cell Death Dis*. 2021; 12 (1): 109. doi: 10.1038/s41419-020-03385-x.
64. *Sayed D, Abdellatif M*. MicroRNAs in development and disease. *Physiol Rev*. 2011; 91 (3): 827–887.
65. *Shevchenko O., Sharapchenko S., Gichkun O., Velikiy D., Tsirulnikova O., Gautier S. et al.* Mir-339 and galectin-3: diagnostic value in patients with airway obstruction after lung transplantation. *Transplant International*. 2021; 3 (9): 1733–1739.
66. *Pérez-Carrillo L, Sánchez-Lázaro I, Triviño JC, Feijóo-Bandín S, Lago F, González-Juanatey JR et al.* Diagnostic value of serum miR-144-3p for the detection of acute cellular rejection in heart transplant patients. *J Heart Lung Transplant*. 2022; 41 (2): 137–147.
67. *Budding K, Rossato M, van de Graaf EA, Kwakkel-van Erp JM, Radstake TRDJ, Otten HG.* Serum miRNAs

as potential biomarkers for the bronchiolitis obliterans syndrome after lung transplantation. *Transpl Immunol*. 2017; 42: 1–4. doi: 10.1016/j.trim.2017.04.002.

68. *Liang J, Tang Y, Liu Z, Wang X, Tang L, Zou Z et al*. Increased expression of miR-155 correlates with abnormal allograft status in solid organ transplant patients and rat kidney transplantation model. *Life Sci*. 2019; 227: 51–57.
69. *Prokop JW, May T, Strong K, Bilinovich SM, Bupp C, Rajasekaran S et al*. Genome sequencing in the clinic: the past, present, and future of genomic medicine. *Physiol Genomics*. 2018; 50 (8): 563–579.
70. *Chau BN, Xin C, Hartner J, Ren S, Castano AP, Linn G et al.* MicroRNA-21 promotes fibrosis of the kidney by silencing metabolic pathways. *Sci Transl Med*. 2012; 4 (121): 121ra18. doi: 10.1126/scitranslmed.3003205.
71. *Denby L, Ramdas V, Lu R, Conway BR, Grant JS, Dickinson B et al.* MicroRNA-214 antagonism protects against renal fibrosis. *J Am Soc Nephrol*. 2014; 25 (1): 65–80.
72. *Chung AC, Huang XR, Meng X, Lan HY.* MiR-192 mediates TGFbeta/Smad3-driven renal fibrosis. *Journal of the American Society of Nephrology*. 2010; 21: 1317–1325.
73. *Wang B, Komers R, Carew R, Winbanks CE, Xu B, Herman-Edelstein M et al.* Suppression of microRNA-29 expression by TGF-β1 promotes collagen expression and renal fibrosis. *J Am Soc Nephrol*. 2012; 23 (2): 252–265.
74. *Oba S, Kumano S, Suzuki E, Nishimatsu H, Takahashi M, Takamori H et al.* miR-200b precursor can ameliorate renal tubulointerstitial fibrosis. *PLoS One*. 2010; 5 (10): e13614. doi: 10.1371/journal.pone.0013614.
75. *Jiang L, Qiu W, Zhou Y, Wen P, Fang L, Cao H et al.* A microRNA-30e/mitochondrial uncoupling protein 2 axis mediates TGF-β1-induced tubular epithelial cell extracellular matrix production and kidney fibrosis. *Kidney Int*. 2013; 84 (2): 285–296.
76. *Li R, Chung AC, Dong Y, Yang W, Zhong X, Lan HY.* The microRNA miR-433 promotes renal fibrosis by amplifying the TGF-β/Smad3-Azin1 pathway. *Kidney Int*. 2013; 84 (6): 1129–1144.

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