FACTA UNIVERSITATIS
Series **Medicine and Biology** – ONLINE FIRST https://doi.org/10.22190/FUMB230406003S

Original Scientific Article

CYTOKINE PROFILE IN RENAL TRANSPLANT PATIENTS IN A LONG-TERM PERIOD AFTER SURGERY

Vladana Stojiljković[1,2], Nikola Stefanović[3], Katarina Danković[3], Branka Đorđević[1], Mina Cvetković[4], Nataša Stević[5], Stevan Vujić[6], Branka Mitić[7,8], Tatjana Cvetković[1,2]

[1]Department of Biochemistry, Faculty of Medicine, University of Niš, Serbia
[2]Center for Medical and Clinical Biochemistry, University Clinical Center Niš, Serbia
[3]Department of Pharmacy, Faculty of Medicine, University of Niš, Serbia
[4]Institute of Cardiology, Deutsches Herzzentrum Berlin, Germany
[5]Faculty of Medicine, University of Niš, Serbia
[6]Center for Biomedical Research, Faculty of Medicine, University of Niš, Serbia
[7]Department of Internal Medicine, Faculty of Medicine, University of Niš, Serbia
[8]Clinic for Nephrology, University Clinical Center Niš, Serbia

Abstract. *In the long-term period after kidney transplantation, a certain level of tissue inflammation and therefore the production of proinflammatory cytokines, including TNF-α, IL-1β, IL-18 and IL-2 can be found. The aim of our study was to determine the concentrations of TNF-α, IL-1β, IL-18, IL-2 and its soluble receptor (IL-2R) in renal transplant patients, regarding the length of the postoperative period. The study involved 65 patients, transplanted at least 12 months prior to our investigation, divided into three groups, regarding the time passed since the transplantation (12-24, 24-48, and >48 months consecutively). Concentrations of the cytokines in the plasma of the subjects were measured using ELISA method. Group I showed significantly higher concentrations of IL-1b compared to the III ($p<0.05$), IL-18 compared to the II and III ($p<0.05$) and TNF-a compared to the II ($p<0.05$). Cytokine concentrations correlated with the time passed since the transplantation ($p<0.05$), except for TNF-a. Interleukin-2 correlated negatively with IL-18 and immunosuppressant dosage ($p<0.05$). Interleukin-1b, IL-18 and TNF-α measurements should be considered for monitoring and detecting potentially subclinical allograft damage in the second year after surgery. However, the dynamics of the change of cytokine concentration may also have been altered by the components of the immunosuppressive protocols used, such as tacrolimus, which is a link that is yet to be examined.*

Key words: *TNF-α, IL-1β, IL-18, IL-2, IL-2 receptor, kidney transplantation*

INTRODUCTION

Kidney transplantation, as a method for the treatment of end-stage chronic kidney disease (CKD) patients, requires a careful modulation of the natural immune response. The transplanted allograft is susceptible to various mechanisms of damage, and even after years of experience in this procedure of treatment, reports state that 10-year allograft survival is around 65% [1]. The 10-year graft survival rate has not changed much since 2005 [2] and the newest Organ Procurement and Transplantation Network report does not state the current percentage for adult recipients [3].

Immunosuppressive protocols for kidney transplant recipients usually include calcineurin inhibitor agents, such as cyclosporine A (CSA) and tacrolimus (TAC), alongside with antiproliferative drug mycophenolate mofetil (MMF) and corticosteroid therapy [4]. Both CSA and TAC inhibit the activation and proliferation of T-cells. Cyclosporine A binds to cyclophilin protein within T-cells [5], while TAC binds to immunophilin FKBP12. The further effect of the complexes that formed is a cascade of blocking calcineurin and an inhibition of several cytokine gene transcriptions, including interleukin 2 (IL-2) [6].

Chronic allograft disfunction and rejection, as a consequence of constant immune damage to the graft, is mainly the result of failure to maintain the level of immunosuppression, necessary to control the reaction of the immune system to allogeneic tissue. Non-immunological factors (factors related to the donor, duration of ischemia, nephrotoxicity of immunosuppressive therapy, arterial hypertension, infections, recurrent or newly

Correspondence to: Vladana Stojiljković
Department of Biochemistry, Faculty of Medicine, University of Niš, Bulevar dr Zorana Djindjića 81, 18000 Niš, Serbia
E-mail: vladana.stojiljkovic@medfak.ni.ac.rs
Received April 6th, 2023 / Revised April 18th, 2023 / Accepted April 18th, 2023

occurring glomerular disease, smoking) contribute to the allograft damage as well [7,8]. A progressive decline in renal function occurs, as well as the invasion of the renal parenchyma by the T-cells. Immunohistology examination of the interstitial tissue shows a predominance of mononuclear cells that are positive for MHC class II molecules and interleukin-2 receptor (IL-2R) [9]. Smooth muscle cell proliferation can also be detected, with the signs of hyperplasia in blood vessels, neointima formation, destruction of the internal elastic lamina, and finally, vascular occlusion [9].

Pathophysiological mechanisms of ongoing graft injury are various. Although the damage caused by ischemia and reperfusion of the graft is usually associated with acute injuries, mostly early after the transplantation, it does play a role in the processes that affect the transplanted organ in the long term as well. Namely, as a result of a reaction of the endothelium to such damage, higher P-selectin expression, causes the binding of polymorphonuclear cells and monocytes to the intima of blood vessels [10]. A similar mechanism of non-specific immune reaction is present in the case of graft damage of another nature, which triggers local production of interleukin 1 beta (IL-1β) and tumor necrosis factor alpha (TNF-α) by leukocytes. This activation of non-specific immunity is of cascade type and constantly increases leukocyte adherence and infiltration of damaged tissue [11].

Apart from the role of circulating cells of the immune system, the role of renal tubular epithelial cells (TEC) as possible immunoregulators is emphasized in the literature [12,13]. Namely, TEC produce various cytokines (IL-6, IL-18, IL-15, TNF-α, TGF-β, chemokines), some of which are pro-inflammatory and some anti-inflammatory [14].

Tumor necrosis factor alpha, IL-1 and IL-18 are inflammatory cytokines, whose high concentrations are observed in biopsies of grafts in acute rejection [15,16]. It has also been shown that TEC in culture, activated by IL-1, secrete TNF-α abundantly [17]. Literature data also indicate that the donor phenotype with higher TNF-α production leads to an increased risk for delayed graft function [18] and rejection [19]. The release of TNF-α induces the synthesis of intercellular adhesion molecule type 1 (ICAM-1) and increases the adhesion of monocytes [20], which then differentiate into macrophages in the tissue and close the vicious circle of TNF-α production in the inflamed tissue.

The increase of IL-1β in urine was reported to be associated with acute rejection, as a sign of increased local production of this cytokine inside the graft and the possible benefit of its serial monitoring in the early postoperative period has been reported [21]. However, there is much less data on the benefits of determining this parameter in the long-term period after transplantation.

A similar distribution in biopsies was observed in the case of IL-18, as well as the fact that this molecule and its receptor are synthesized by TEC cultures when activated by pro-inflammatory cytokines (TNF-α and IFN-γ). By examining TEC cultures, it was also observed that this molecule is an autocrine factor for modulating the function and activity of the TEC cells themselves [22, 23]. These data indicate that IL-18 may play an important role in allograft rejection by stimulating leukocyte infiltration and the immune activity of TECs.

One of the most highlighted cytokines in the process of T lymphocyte activation and proliferation is IL-2. Secreted by the T cells themselves, it activates them back in an autocrine and paracrine way. This effect is achieved through binding to a specific receptor (IL-2R) and the formation of IL2-IL2R complex that starts the cell signaling cascade [24].

The existing diagnostic algorithm for monitoring kidney transplant patients is based on monitoring renal function by determining creatinine clearance, however, in about 30% of clinically stable transplant patients, without deterioration of graft function, histological signs of rejection can be found [2]. These subclinical rejection reactions can be detected by repeated analyses of allograft biopsies obtained by protocol biopsies [25], which represent invasive diagnostic procedures, and the discovery of new, reliable and more accessible markers would be of great benefit in timely response to subclinical allograft damage.

Thus, the aim of this study was to determine concentrations of TNF-α, IL-18, IL-1β and IL-2 and its soluble receptor fraction in patients in a long-term period after kidney transplantation.

MATERIALS AND METHODS

The research was conducted in the Laboratory of the Institute of Biochemistry, Faculty of Medicine, University of Nis and at the Clinic for Nephrology, University Clinical Center Nis. The study protocol was approved by the Ethics Committees of the Faculty of Medicine, University of Nis, Nis, Serbia (approval no. 01-10204-13). The study was conducted in accordance with the Declaration of Helsinki and the instructions for good clinical practice. Written informed consent for participation in the study was obtained from all study participants.

The study included 65 kidney transplant patients, at different periods after transplantation, divided into 3 groups:

1. Patients with transplant 12-24 months (15 patients);
2. Patients with transplant 24-48 months (20 patients);
3. Patients with a transplant longer than 48 months (30 patients).

Most of the patients had TAC- based immunosuppressive protocol (56 patients), while the rest (9 patients) received CSA as immunosuppressant.

The research used plasma and serum obtained from a venous blood sample. The glomerular filtration rate was estimated (eGFR) based on the MDRD formula:

$$eGFR\ (mL/min/1.73\ m^2) = 32788 \times (creatinine\ in\ \mu mol\ /\ L)^{-1.154} \times (Years\ of\ age)^{-0.203} \times$$
$$\times\ (0.742\ for\ women) \times (1.212\ for\ black\ race\ subjects)$$

Concentrations of serum creatinine and urea were determined using standard methods on an automatic analyzer (AU680 Clinical Chemistry Analyzer, Beckman Coulter, Brea, CA, USA).

Concentrations of the examined cytokines in the subjects' plasma were measured by the ELISA technique, using commercially available kits:

- ELISA Kit for human IL-18 (MBL International Corporation, MDD=12.5 pg/ml);
- Quantikine® Colorimetric Sandwich ELISAs hsIL-1β (high sensitivity; R&D Systems, MDD=0.063 pg/ml);
- Quantikine® Colorimetric Sandwich ELISAs TNF-α (R&D Systems, MDD=6.23 pg/ml);
- Quantikine® Colorimetric Sandwich ELISAs IL-2 (R&D Systems, MDD=7 pg/ml);
- ELISA kit for human IL-2 receptor (Abcam, MDD=68.75 pg/ml).

Optical density of the performed ELISA tests was determined at the wavelength stated in the kit protocol using Thermo Scientific™ Multiskan™ FC Microplate Photometer (Thermo Fisher Scientific).

To compare the obtained results between the groups the ANOVA for unpaired samples (with normal distribution of parameter values) or the Kruskal-Wallis test (when the normal distribution of the values is not satisfied) was used. Correlation analysis included Spearman's correlation coefficient calculation for non-normally distributed data. Statistical analysis was performed using the SPSS software package (version 25) at a significance level of $p < 0.05$.

RESULTS

The groups we examined in our study did not differ in terms of age and gender structure (Table 1).

Table 1 Demographic characteristics of the examined patient groups.

	I group (12-24 months after transplantation)	II group (24-48 months after transplantation)	III group (more than 48 months after transplantation)	Significance (p)
Age (years)	42.2 ± 15.09	41.6 ± 9.05	44.2 ± 9.81	p>0.05
Sex (M/F)	8/7	12/8	14/16	p>0.05
Serum creatinine concentration (sCRE) (μmol / L)	191.873 ± 41.05	148.74 ± 65.31	134.11 ± 28.29	I vs II: p=0.03 I vs III: p<0.0001
Serum urea concentration (sUrea) (mmol / L)	11.06 ± 8.07	9.59 ± 5.95	9.52 ± 5.36	I vs III: p<0.0001
eGFR ($mL/min/1.73m^2$)	42.72 ± 16.96	48.47 ± 14.19	48.09 ± 17.48	p>0.05

Data presented as mean ± SD or *n* of participants. ANOVA test was used to compare the means between the groups.

The results showed statistically significant differences in values of IL-1β, IL-18 and TNF-α among the groups of patients, while IL-2 and IL-2R differences were non-significant (Table 2). Group I had significantly higher concentrations of IL-1β compared to the III group ($p<0.05$). A similar finding occurred in the case of IL-18 as well, in which case I group had higher values compared to both II and III group ($p<0.05$). Tumor necrosis factor alpha turned out to be the highest in concentration in the I group, significantly compared to the II one ($p<0.05$).

Table 2 Cytokine concentration difference between the examined groups of patients.

Cytokine	I group	II group	III group	Significance (p)
IL-2 (pg/ml)	8.07±6.68	9.33±5.49	9.26±3.96	p>0.05*
IL-2R (pg/ml)	1231.18±730.4	1434.09±400.0	1700.75±1035.74	p>0.05*
IL-1β (pg/ml)	1.48±1.02	1.15±0.51	0.79±0.2	I vs III p<0.05*
IL-18 (pg/ml)	3412.83±1384.95	2389.9±1134.53	2578.69±1058.13	I vs II, I vs III $p<0.05^{\#}$
TNF-α (pg/ml)	7.46±3.83	4.33±1.99	6.3±2.2	I vs II p<0.05*

Data presented as mean ± SD. '#': ANOVA test was used to compare the means between the groups of normally distributed data. '*': Kruskal Wallis test was used if data were not normally distributed. Statistical analysis was done at the significance level of $p<0.05$.

The correlation analysis we conducted included dosage and the concentration of the immunosuppressant drug used (TAC or CSA), as well as the time passed since the transplantation. The correlation was significant between years since the transplantation and all other parameters examined ($p<0.05$), except for TNF-α and IL-2R. These

correlations were negative between the time passed since the transplantation and IL-18, IL-1β and dosage of immunosuppressant, whereas IL-2 and immunosuppressant concentration correlated positively (Table 3). Interleukin 2 correlated negatively with IL-18 and immunosuppressant dosage ($p<0.05$).

Table 3 Correlation analysis between the cytokine concentrations and additional factors.

	IL-18	IL-2	IL-2R	IL-1β	TNF-α	TAC/CSA dose	TAC/CSA concentration
Time since the transplantation	-0.292 (0.018)*	0.261 (0.036)*	0.233 (0.062)	-0.320 (0.009)*	-0.145 (0.249)	-0.257 (0.039)*	0.347 (0.005)*
IL-18		-0.261 (0.036)*	-0.070 (0.578)	0.041 (0.744)	0.080 (0.526)	0.201 (0.108)	-0.076 (0.549)
IL-2			0.055 (0.665)	-0.114 (0.366)	-0.083 (0.512)	-0.315 (0.011)*	-0.183 (0.145)
IL-2R				-0.049 (0.700)	0.023 (0.854)	0.213 (0.089)	0.151 (0.229)
IL-1β					-0.170 (0.175)	-0.020 (0.873)	-0.165 (0.188)
TNF-α						0.040 (0.754)	0.072 (0.569)
TAC/CSA dose							-0.086 (0.498)

Data presented as correlation coefficient (Spearman's) including significance (p). $p<0.05$ was considered statistically significant. *: $p<0.05$.

DISCUSSION

In the long-term period after transplantation, promptly ongoing processes, such as acute and per acute rejection of the graft, are less important, while damage to the graft by other mechanisms, such as oxidative stress, chronic inflammation and vascular damage, receive greater attention in terms of loss of organ function if they are not recognized on time. One of the more prevalent processes is chronic allograft nephropathy (CAN), the pathogenesis of which is still not well understood, however, inflammation, mediated by specific (chronic rejection reaction) and non-specific (drug toxicity, ischemia) mechanisms, remain constant characteristic of this process [7, 26]. Macrophage infiltration is a feature of alloimmunity in general, but may be intensified by the ongoing stimuli of a different nature, such as ischemia [7, 27]. Experimental data from animal model based experiments indicate that the intensity of macrophage infiltration of the allograft after episodes of acute rejection or acute graft damage of another nature could be a predictive factor for assessing the risk of developing CAN in the later period [28]. One group of researchers, also using an animal model, proved that the application of inhibitors of macrophage function, adenoviral IL-10 and antagonists of TNF-α and IL-12 (macrophage products), stops the development of clinical and histological changes in CAN [29]. Pilmore et al, in their study with protocol biopsies of allografts in humans, concluded that the intensity of macrophage infiltration in early biopsies was predictive of later development of CAN [30]. The mechanisms of macrophage participation in the development of CAN are complex, but the proinflammatory effect mediated by cytokines (TNF-α, IL-1) and cell damage by products of oxidative stress reactions are mentioned as probable mechanisms [31, 32].

Activated macrophages synthesize a large number of proinflammatory cytokines including IL-1, IL-18, and TNF-α [32, 33]. Interleukin 1 activates endothelial cells and induces the production of other cytokines, chemokines, and leukocyte adhesion molecules [34]. Messenger RNA (mRNA) for IL-1, as well as IL-1 is increased in the rejection reaction, and recipients with the appropriate phenotype who produce large amounts of IL-1 receptor antagonists are less prone to allograft rejection [35]. Increased serum and tissue TNF-α, as well as increased TNF-α mRNA levels in graft tissue have been associated with acute renal and cardiac graft rejection [36, 37].

The results of our research show that the level of TNF-α is the highest in the first group of patients, the most recently transplanted patients. This result points to the possibility of increased vulnerability of the graft to the immune response of the recipient in the earlier period compared to the later years following transplantation. However, the initial pathology that led to the transplantation itself, as well as the changes and comorbidities that occurred during the hemodialysis that preceded the transplantation should be taken into account. Namely, it has been published that there are significant changes in macrophage activity and production of TNF-α and IL-1 in hemodialysis patients, which was significantly increased [38]. In accordance with this, the influence of long-term hemodialysis on the production of pro-inflammatory cytokines in these patients should be notified.

A similar study also looked at the dynamics of changes in IL-1β concentration in hemodialysis patients, which was also significantly higher compared to controls [39]. The level of this cytokine also showed variations during the hemodialysis procedure itself, which may indicate the value of this parameter in the earlier detection of

macrophage function activation [40]. Our results showed variations in the level of IL-1β in all examined groups, however, only the decrease in the concentration of this cytokine in the third group compared to the first group of patients was significant. We can associate this finding with the relatively stable state of tissue and allograft function in patients who potentially spent the same amount of time on hemodialysis and with the functioning transplant. Also, this is the group of patients who have been on immunosuppressive therapy for the longest time, with protocols that include CSA and TAC, whose mechanism of action is partially based on the suppression of the synthesis of proinflammatory cytokines, including TNF-α and IL-1. Squadrito et al examined one of the animal models of arterial occlusive shock and showed that TAC treatment reduces the level of circulating TNF-α, the expression of adhesion molecules on blood vessels and leukocytes and tissue infiltration by leukocytes, and concluded that the TAC inhibition of TNF-α has a vasculo-protective effect in this model of ischemia-reperfusion damage [41]. Accordingly, the role of immunosuppressive therapy on the level of these parameters should also be considered.

Systemic neutralization of TNF-α activity significantly prolongs allograft maintenance in experimental primate models [42] and reduces kidney injury in the graft ischemia-reperfusion (IRI) model [43]. All these studies suggest that TEC, which produce TNF-α, can play an important effector role in kidney allograft rejection by producing this cytokine within the graft, which can later either damage the graft cells directly or indirectly, by activating leukocytes that infiltrate the tissue.

The results of our study showed significantly higher concentration of plasma IL-18 in the first group compared to the second and third. In the literature, there are data on the possible role of IL-18, originating from macrophages in renal allograft rejection [44]. These studies also showed a reduction in allograft damage in macrophage-depleted animals, which then showed significantly lower levels of IL-18 [37]. The receptor for IL-18 is also expressed on TEC and undergoes up-regulation in response to TNF-α and other pro-inflammatory cytokines [23], suggesting the possibility that IL-18 produced by TEC can autocrinally regulate their activity, which some studies have shown on TEC cultures [45, 46]. These data indicate that IL-18 may play an important role in renal allograft rejection by stimulating both leukocyte infiltration and activation of residual and TEC. However, in a fully MHC-incompatible murine model of acute renal rejection, neither IL-18 deficiency from recipient leukocytes nor systemic neutralization of IL-18 activity showed a protective effect in the allograft rejection reaction [47]. This finding suggests that IL-18 may only have a minimal effect in renal allograft rejection, as a co-stimulatory cytokine.

In this study, IL-1β and IL-18 showed a significant negative correlation with the years passed since the transplantation, while the correlation of IL-2 and IL-2R was positive. However, since IL-2 and IL-2R did not differ significantly among the groups of patients, this correlation should be interpreted carefully. Other studies also made different conclusions on this matter. Some proved favorable outcomes when IL-2R antibody treatment is applied [48], while some reported that there were no significant effects [49, 50]. Generally, in protocols using TAC as an immunosuppressive agent, IL-2R antibody does not seem to have benefits, and in order to get more precise conclusions, more randomized trials should be conducted [51].

Our results also showed a significant negative correlation between IL-2 and IL-18. Although IL-2 and IL-18 do not have a direct functional connection, they can interact indirectly through their effects on other immune cells and cytokines. For example, IL-2 can stimulate the production of IFN-γ, which in turn can activate macrophages and enhance antigen presentation, leading to an enhanced immune response. Similarly, IL-18 can stimulate the production of IFN- γ and other cytokines, which can activate T and NK cells and promote an immune response against infections and tumors [52]. The negative correlation obtained in this study could be a result of treatment effects to IL-2 production, since there was a negative correlation with the immunosuppressant dosage as well. However, since the correlation with the concentration of the drug did not prove to be statistically significant, the explanation is most likely the number of participants we included.

The study we conducted has some limitations that might have influenced the results we obtained. The patient group we examined had 65 participants, with majority of them being treated with TAC based immunosuppression protocol. It would be advisable to expand the examined cohort and include more CSA treated patients, as well as multiple cytokine measurements during post-transplant follow up period in order to draw more precise conclusions.

CONCLUSION

In this study, patients 12-24 months after kidney transplantation had significantly higher concentrations of IL-1β, IL-18 and TNF-α compared to the rest of the patients who received the allograft 24-48 and more than 48 months prior to the beginning of this research. All examined cytokines, except TNF-α, correlated with the time passed since the transplantation.

Accordingly, IL-1β, IL-18 and TNF-α concentrations should be considered for monitoring and detecting potentially subclinical allograft damage in the second year after surgery. Due to their mechanism of action, drugs from the immunosuppressive protocols used in the therapy of these patients, such as TAC, participate in the modulation of the synthesis of pro-inflammatory cytokines, IL-18 and IL-2 included, thus affecting their concentration. Therefore, this influence should be observed more carefully, preferably in a larger cohort, in order to explain negative correlations such as the one we found between IL-2 and IL-18.

ACKNOWLEDGEMENTS. *This work was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia* (*project no. 451-03-9/2022-14/200113).*

REFERENCES

1. Ghelichi-Ghojogh M, Ghaem H, Mohammadizadeh F, et al. Graft and Patient Survival Rates in Kidney Transplantation, and Their Associated Factors: A Systematic Review and Meta-Analysis. Iran J Public Health 2021; 50(8):1555–63. https://pubmed.ncbi.nlm.nih.gov/34917526/
2. Hart A, Smith JM, Skeans MA, et al. OPTN/SRTR 2015 Annual Data Report: Kidney. Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg 2017; 17 Suppl 1(Suppl 1):21–116. https://pubmed.ncbi.nlm.nih.gov/28052609/
3. Lentine KL, Smith JM, Miller JM, et al. OPTN/SRTR 2021 Annual Data Report: Kidney. Am J Transplant 2023; 23(2):S21–120. https://pubmed.ncbi.nlm.nih.gov/35266618/
4. Rummo O, Carmellini M, Kamar N, et al. Long-term, prolonged-release tacrolimus-based immunosuppression in de novo kidney transplant recipients: 5-year prospective follow-up of the ADHERE study patients. Transpl Int 2020; 33(2):161–73. https://pubmed.ncbi.nlm.nih.gov/31536654/
5. Russell G, Graveley R, Seid J, al-Humidan AK, Skjodt H. Mechanisms of action of cyclosporine and effects on connective tissues. Semin Arthritis Rheum 1992; 21(6 Suppl 3):16–22. https://pubmed.ncbi.nlm.nih.gov/1502562/
6. Thomson AW, Bonham CA, Zeevi A. Mode of action of tacrolimus (FK506): molecular and cellular mechanisms. Ther Drug Monit 1995; 17(6):584–91. https://pubmed.ncbi.nlm.nih.gov/8588225/
7. Andrian T, Siriteanu L, Covic AS, et al. Non-Traditional Non-Immunological Risk Factors for Kidney Allograft Loss—Opinion. J Clin Med 2023; 12(6):2364. https://pubmed.ncbi.nlm.nih.gov/36983364/
8. Van Loon E, Bernards J, Van Craenenbroeck AH, Naesens M. The Causes of Kidney Allograft Failure: More Than Alloimmunity. A Viewpoint Article. Transplantation 2020; 104(2):e46. https://pubmed.ncbi.nlm.nih.gov/32000235/
9. Solez K, Colvin RB, Racusen LC, et al. Banff 07 classification of renal allograft pathology: updates and future directions. Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg 2008; 8(4):753–60. https://pubmed.ncbi.nlm.nih.gov/18294345/
10. Takada M, Nadeau KC, Shaw GD, Marquette KA, Tilney NL. The cytokine-adhesion molecule cascade in ischemia/reperfusion injury of the rat kidney. Inhibition by a soluble P-selectin ligand. J Clin Invest 1997; 99(11):2682–90. https://pubmed.ncbi.nlm.nih.gov/9169498/
11. Fernández AR, Sánchez-Tarjuelo R, Cravedi P, Ochando J, López-Hoyos M. Review: Ischemia Reperfusion Injury—A Translational Perspective in Organ Transplantation. Int J Mol Sci 2020; 21(22):8549. https://pubmed.ncbi.nlm.nih.gov/33202744/
12. Li Y, Yang J, Luo JH, Dedhar S, Liu Y. Tubular Epithelial Cell Dedifferentiation Is Driven by the Helix-Loop-Helix Transcriptional Inhibitor Id1. J Am Soc Nephrol 2007; 18(2):449. https://pubmed.ncbi.nlm.nih.gov/17202424/
13. Kooten C van, Daha MR, Es LA van. Tubular Epithelial Cells: A Critical Cell Type in the Regulation of Renal Inflammatory Processes. Nephron Exp Nephrol 1999; 7(5–6):429–37. https://pubmed.ncbi.nlm.nih.gov/10559641/
14. Smith SF, Hosgood SA, Nicholson ML. Ischemia-reperfusion injury in renal transplantation: 3 key signaling pathways in tubular epithelial cells. Kidney Int 2019; 95(1):50–6. https://pubmed.ncbi.nlm.nih.gov/30606429/
15. Hribova P, Kotsch K, Brabcova I, Vitko S, Volk HD, Lacha J. Cytokines and Chemokine Gene Expression in Human Kidney Transplantation. Transplant Proc 2005; 37(2):760–3. https://pubmed.ncbi.nlm.nih.gov/15848523/
16. Kaminska D, Tyran B, Mazanowska O, et al. Cytokine Gene Expression in Kidney Allograft Biopsies After Donor Brain Death and Ischemia-Reperfusion Injury Using In Situ Reverse-Transcription Polymerase Chain Reaction Analysis. Transplantation 2007; 84(9):1118. https://pubmed.ncbi.nlm.nih.gov/17998866/
17. Nguan CYC, Du C. Renal tubular epithelial cells as immunoregulatory cells in renal allograft rejection. Transplant Rev 2009; 23(3):129–38. https://pubmed.ncbi.nlm.nih.gov/19361977/
18. Alakulppi NS, Kyllönen LE, Jäntti VT, et al. Cytokine Gene Polymorphisms and Risks of Acute Rejection and Delayed Graft Function after Kidney Transplantation. Transplantation 2004; 78(10):1422. https://pubmed.ncbi.nlm.nih.gov/15599305/
19. Gandhi N, Goldman D, Kahan D, et al. Donor cytokine gene polymorphisms are associated with increased graft loss and dysfunction after transplant. Transplant Proc 2001; 33(1–2):827–8. https://pubmed.ncbi.nlm.nih.gov/11267084/
20. Timoshanko JR, Sedgwick JD, Holdsworth SR, Tipping PG. Intrinsic Renal Cells Are the Major Source of Tumor Necrosis Factor Contributing to Renal Injury in Murine Crescentic Glomerulonephritis. J Am Soc Nephrol 2003; 14(7):1785. https://pubmed.ncbi.nlm.nih.gov/12819238/
21. Sarhan M, von Mässenhausen A, Hugo C, Oberbauer R, Linkermann A. Immunological consequences of kidney cell death. Cell Death Dis 2018; 9(2):114. https://pubmed.ncbi.nlm.nih.gov/29371597/
22. Striz I, Krasna E, Eliska K, et al. Interleukin 18 (IL-18) upregulation in acute rejection of kidney allograft. Immunol Lett 2005; 99:30–5. https://pubmed.ncbi.nlm.nih.gov/15894108/
23. Krásná E, Kolesár L, Slavčev A, et al. IL-18 Receptor Expression on Epithelial Cells is Upregulated by TNF Alpha. Inflammation 2005; 29(1):33–7. https://pubmed.ncbi.nlm.nih.gov/16502344/
24. Park SJ, Yoon YC, Kang SW, et al. Impact of IL2 and IL2RB Genetic Polymorphisms in Kidney Transplantation. Transplant Proc 2011; 43(6):2383–7. https://pubmed.ncbi.nlm.nih.gov/21839273/
25. Fu MS, Lim SJ, Jalalonmuhali M, Ng KS, Lim SK, Ng KP. Clinical Significance of Renal Allograft Protocol Biopsies: A Single Tertiary Center Experience in Malaysia. J Transplant 2019; 2019:e9153875. https://pubmed.ncbi.nlm.nih.gov/31186948/
26. Sanjay S. Comparative study of once daily tacrolimus (extended-release capsule) versus conventional twice daily tacrolimus in renal transplant recipients. Int J Clin Virol 2022; 6(2):050–4. https://www.heighpubs.org/hjcv/ijcv-aid1050.php
27. Herrero-Fresneda I, Torras J, Cruzado JM, et al. Do Alloreactivity and Prolonged Cold Ischemia Cause Different Elementary Lesions in Chronic Allograft Nephropathy? Am J Pathol 2003; 162(1):127–37. https://pubmed.ncbi.nlm.nih.gov/12507896/
28. Shimizu A, Yamada K, Sachs DH, Colvin RB. Mechanisms of Chronic Renal Allograft Rejection. II. Progressive Allograft Glomerulopathy in Miniature Swine. Lab Invest 2002; 82(6):673–86. https://pubmed.ncbi.nlm.nih.gov/12065677/
29. Yang J, Reutzel-Selke A, Steier C, et al. Targeting of macrophage activity by adenovirus-mediated intragraft overexpression of TNFRp55-Ig, IL-12p40, and vIL-10 ameliorates adenovirus-mediated chronic graft injury, whereas stimulation of macrophages by overexpression of IFN-gamma accelerates chronic graft injury in a rat renal allograft model. J Am Soc Nephrol JASN 2003; 14(1):214–25. https://pubmed.ncbi.nlm.nih.gov/12506154/
30. Pilmore HL, Painter DM, Bishop GA, McCaughan GW, Eris JM. Early up-regulation of macrophages and myofibroblasts: a new marker for development of chronic renal allograft rejection. Transplantation 2000; 69(12):2658–62. https://pubmed.ncbi.nlm.nih.gov/10910290/
31. Savikko J, Taskinen E, Von Willebrand E. Chronic allograft nephropathy is prevented by inhibition of platelet-derived growth factor receptor: tyrosine kinase inhibitors as a potential therapy. Transplantation 2003; 75(8):1147–53. https://pubmed.ncbi.nlm.nih.gov/12717194/
32. Zhang H, Li Z, Li W. M2 Macrophages Serve as Critical Executor of Innate Immunity in Chronic Allograft Rejection. Front Immunol 2021; 12:648539. https://pubmed.ncbi.nlm.nih.gov/33815407/

33. Devraj VM, Kalidindi K, Guditi S, Uppin M, Taduri G. Macrophage polarization in kidney transplant patients. Transpl Immunol 2022; 75:101717. https://pubmed.ncbi.nlm.nih.gov/36130699/
34. Yazısız V, Yılmaz VT, Uçar İ, et al. The use of anti-interleukin-1 agents and tumor necrosis factor-alpha inhibitors in renal transplant recipients. Arch Rheumatol 2021; 36(3):366–74. https://pubmed.ncbi.nlm.nih.gov/34870168/
35. Mulders-Manders CM, Baas MC, Molenaar FM, Simon A. Peri- and Postoperative Treatment with the Interleukin-1 Receptor Antagonist Anakinra Is Safe in Patients Undergoing Renal Transplantation: Case Series and Review of the Literature. Front Pharmacol 2017; 8:342. https://pubmed.ncbi.nlm.nih.gov/28620307/
36. Rowshani AT, Vereyken EJF. The Role of Macrophage Lineage Cells in Kidney Graft Rejection and Survival. Transplantation 2012; 94(4):309. https://pubmed.ncbi.nlm.nih.gov/22828735/
37. Wyburn KR, Jose MD, Wu H, Atkins RC, Chadban SJ. The Role of Macrophages in Allograft Rejection. Transplantation 2005; 80(12):1641. https://pubmed.ncbi.nlm.nih.gov/16378052/
38. Eloueyk AK, Alameddine RY, Osta BA, Awad DM. Correlations between serum inflammatory markers and comorbidities in patients with end-stage renal disease. J Taibah Univ Med Sci 2019; 14(6):547–52. https://pubmed.ncbi.nlm.nih.gov/31908643/
39. Hung AM, Ellis CD, Shintani A, Booker C, Ikizler TA. IL-1β Receptor Antagonist Reduces Inflammation in Hemodialysis Patients. J Am Soc Nephrol 2011; 22(3):437. https://pubmed.ncbi.nlm.nih.gov/21310819/
40. Herbelin A, Nguyen AT, Zingraff J, Ureña P, Descamps-Latscha B. Influence of uremia and hemodialysis on circulating interleukin-1 and tumor necrosis factor α. Kidney Int 1990; 37(1):116–25. https://pubmed.ncbi.nlm.nih.gov/2299797/
41. Squadrito F, Altavilla D, Squadrito G, et al. Tacrolimus suppresses tumour necrosis factor-α and protects against splanchnic artery occlusion shock. Br J Pharmacol 1999; 127(2):498–504. https://pubmed.ncbi.nlm.nih.gov/10385251/
42. Li J, Li C, Zhuang Q, et al. The Evolving Roles of Macrophages in Organ Transplantation. J Immunol Res 2019; 2019:e5763430. https://pubmed.ncbi.nlm.nih.gov/31179346/
43. Malek M, Nematbakhsh M. Renal ischemia/reperfusion injury; from pathophysiology to treatment. J Ren Inj Prev 2015; 4(2):20–7. https://pubmed.ncbi.nlm.nih.gov/26060833/
44. Li J, Nozaki Y, Akazawa H, Kishimoto K, Kinoshita K, Matsumura I. Deletion of Antigen-Presenting Cells in Lipopolysaccharide-Induced Acute Kidney Injury (AKI) Affects the Exacerbation and Repair in AKI. Curr Issues Mol Biol 2022; 44(11):5655–65. https://pubmed.ncbi.nlm.nih.gov/36421667/
45. Liang D, Liu HF, Yao CW, et al. Effects of interleukin 18 on injury and activation of human proximal tubular epithelial cells. Nephrology 2007; 12(1):53–61. https://pubmed.ncbi.nlm.nih.gov/17295661/
46. Thomas JM, Ling YH, Huuskes B, et al. IL-18 (Interleukin-18) Produced by Renal Tubular Epithelial Cells Promotes Renal Inflammation and Injury During Deoxycorticosterone/Salt-Induced Hypertension in Mice. Hypertension 2021; 78(5):1296–309. https://pubmed.ncbi.nlm.nih.gov/34488433/
47. Wyburn K, Wu H, Chen G, Yin J, Eris J, Chadban S. Interleukin-18 Affects Local Cytokine Expression But Does Not Impact on the Development of Kidney Allograft Rejection. Am J Transplant 2006; 6(11):2612–21. https://pubmed.ncbi.nlm.nih.gov/17049054/
48. Willoughby LM, Schnitzler MA, Brennan DC, et al. Early outcomes of thymoglobulin and basiliximab induction in kidney transplantation: Application of statistical approaches to reduce bias in observational comparisons. Transplantation 2009; 87(10):1520–9. https://pubmed.ncbi.nlm.nih.gov/19461489/
49. Lim WH, Chadban SJ, Campbell S, Dent H, Russ GR, Mcdonald SP. Interleukin-2 receptor antibody does not reduce rejection risk in low immunological risk or tacrolimus-treated intermediate immunological risk renal transplant recipients. Nephrology 2010; 15(3):368–76. https://pubmed.ncbi.nlm.nih.gov/19935375/
50. Tanriover B, Zhang S, MacConmara M, et al. Induction Therapies in Live Donor Kidney Transplantation on Tacrolimus and Mycophenolate With or Without Steroid Maintenance. Clin J Am Soc Nephrol 2015; 10(6):1041. https://pubmed.ncbi.nlm.nih.gov/25979971/
51. Ali H, Mohiuddin A, Sharma A, et al. Implication of interleukin-2 receptor antibody induction therapy in standard risk renal transplant in the tacrolimus era: a meta-analysis. Clin Kidney J 2019; 12(4):592–9. https://pubmed.ncbi.nlm.nih.gov/31384453/
52. Rodriguez-Galán MC, Bream JH, Farr A, Young HA. Synergistic Effect of IL-2, IL-12, and IL-18 on Thymocyte Apoptosis and Th1/Th2 Cytokine Expression12. J Immunol 2005; 174(5):2796–804. https://pubmed.ncbi.nlm.nih.gov/15728489/