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AGE-RELATED FEATURES OF THE PATTERN OF LYMPHOCYTE SUBPOPULATIONS AND FUNCTIONAL ACTIVITY OF PERIPHERAL BLOOD MONONUCLEAR CELLS IN PATIENTS WITH CHRONIC KIDNEY DISEASE BEFORE AND AFTER TRANSPLANTATION

D.V. Artemov, A.B. Zulkarnaev, A.V. Vatazin

Vladimirsky Moscow Regional Research and Clinical Institute, Moscow, Russian Federation

Objective: to analyze the features of the pattern of lymphocyte subpopulations and the functional activity of peripheral blood mononuclear cells in older adult patients with chronic kidney disease. **Materials and methods.** The study featured 21 patients with chronic kidney disease (CKD), over 55 years of age, who underwent kidney transplantation (KT) from unrelated suboptimal donors. The average age was 61.4 ± 4.5 years (55 to 69). Comorbidity was assessed using the CIRS-G scale; the average number of points was 13.6 ± 5.09. The control group consisted of 21 volunteers, aged 55–70, without acute inflammatory diseases and signs of chronic kidney disease (CKD). The average age was 61.1 ± 4.4 years, the average CIRS-G score was 12.11 ± 6.04. In all patients, the pattern of lymphocyte subpopulations of peripheral blood was evaluated by flow cytometry. Vital computer laser cytomorphometry was used to assess the functional state of peripheral blood mononuclear cells. The Functional Activities Index (FAI) was evaluated to indirectly assess the degree of functional activity of cells. **Results.** In CKD patients before KT, there was a decrease in the proportion of CD4 cells ($p = 0.009$), an increase in the proportion of CD8 cells ($p = 0.02$), a decrease in the CD4/CD8 ratio ($p = 0.017$), an increase in the proportion of natural killers ($p = 0.025$) compared with healthy volunteers. Moreover, a decrease in the total proportion of CD3 cells, an increase in HLA-DR expression on CD3 cells, and an increase in the proportion of B cells were statistically insignificant: $p = 0.137$, $p = 0.072$ and $p = 0.135$, respectively. On the fifth day after KT, the proportion of CD3 cells increased ($p = 0.017$) mainly due to an increase in the proportion of CD4 cells ($p = 0.002$) compared to the pre-KT index. The proportion of natural killers ($p = 0.002$) and HLA-DR expression on CD3 cells ($p < 0.0001$) also increased. An increase in the proportion of CD8 cells and in the CD4/CD8 ratio, and a decrease in the proportion of B cells were statistically insignificant: $p = 0.439$, $p = 0.277$, and $p = 0.236$, respectively. A decrease in FAI was noted in patients with CKD before KT in comparison with healthy volunteers ($p = 0.0138$). After ATP, this indicator significantly increased compared to the pre-KT value ($p < 0.0001$) and exceeded the FAI value in healthy volunteers ($p < 0.0001$). In healthy volunteers, there was no significant correlation between the functional activity of peripheral blood mononuclear cells and age ($r = –0.263$ [95% CI –0.6236; 0.1907], $p = 0.264$, $r^2 = 0.069$). At the same time, significant negative correlation between FAI and age was noted in CKD patients: $r = –0.52$ [95% CI –0.7771; –0.1135], $p = 0.0157$, $r^2 = 0.27$ before KT; $r = –0.418$ [95% CI –0.7559; –0.06256], $p = 0.0272$, $r^2 = 0.175$ after KT. **Conclusion.** Older adult CKD patients before and after KT were likely to have significant changes in the morphofunctional state of peripheral blood mononuclear cells and pattern of lymphocyte subpopulations. Moreover, the severity of changes in the functional state of these cells had a strong correlation with age, which was not observed in the group of healthy volunteers. This should be considered when choosing immunosuppressive therapy in older kidney transplant recipients.

Keywords: chronic kidney disease, kidney transplantation, lymphocyte subpopulations, cell functional activity.

INTRODUCTION

The number of patients with stage 5 chronic kidney disease (CKD) is steadily growing, as evidenced by reports from broad professional communities both in Russia and abroad. This is caused by a significant increase in the availability of renal replacement therapy, the increasing prevalence of diseases manifested by renal failure, as well as significant improvements in quality of the current renal replacement therapy which increases the life expectancy in this category of patients [1–3].

Among all methods of renal replacement therapy, optimal is cadaver kidney allotransplantation (CKAT) which provides the best medical and social rehabilitation, life quality and life expectancy in patients [1–3]. The growing shortage of donor organs has led to revision of the principles of their distribution. At present, fun-

Corresponding author: Dmitriy Artemov. Address: Building 6, 61/2, Shchepkina str., Moscow, 129110, Russian Federation. Tel. (926) 528-96-52. E-mail: anatom555@yandex.ru

damental is their quality [4]. The main way to increase the number of donor organs, including kidneys, is to use organs obtained from suboptimal (marginal) donors, e.g. advanced criteria donors (unstable hemodynamics, diabetes mellitus, hypertension, trauma, age-related donors, etc.) [5–7].

An important problem is kidney transplantation to "age-related" recipients. Compared to young, the recipients of kidney allografts of older age groups are featured by the presence of an unfavorable premorbid background associated with the high rate of concomitant chronic diseases (arterial hypertension, coronary heart disease, heart failure, diabetes mellitus, chronic anemia, etc.) and a high polymorbidity index. Clinical manifestations and complications of chronic kidney disease are disguised as a therapeutic symptom complex that determines diseases of the internal organs. This makes transplantation particularly difficult in this group and stresses the importance of choosing the optimal immunosuppressive therapy.

The practical implementation of the "old for old" principle results in this category of patients having better chances of receiving an organ from a suboptimal donor than younger patients. Besides, the features of immunity in elderly patients should be considered when choosing an adequate immunosuppressive therapy [8–11]. There is no doubt that it is the choice of the optimal immunosuppressive therapy that plays the leading role in the long duration of the graft functioning and the recipient's life. In this regard, the study of various aspects of the age-related features of immune homeostasis in patients who are awaiting kidney transplantation seems an important and urgent issue.

Purpose: to analyze the features of the pattern of lymphocyte subpopulations and the functional activity of peripheral blood mononuclear cells in older adult patients with chronic kidney disease.

MATERIALS AND METHODS

The study included 21 patients with chronic kidney disease (CKD) over 55 years of age who underwent kidney transplantation (KT) in 2010–2018. The average age was 61.4 ± 4.5 years (55–69). CKD causes were glomerulonephritis, 23.8% (5 patients); pyelonephritis, 23.8% (5 patients); hypertension, 23.8% (5 patients); diabetes mellitus. 19% (4 patients); polycystic kidney disease, 9.5% (2 patients). Comorbidity was assessed by the Cumulative illness Rating Scale for Geriatrics (CIRS-G) scale; the average score was 13.6 ± 5.09.

All patients received renal replacement therapy, 19 patients – hemodialysis, 2 patients – peritoneal dialysis.

In compliance with the "old for old" principle [13, 14], all donor kidneys were obtained from suboptimal donors: "aged" and "asystolic" donors: categories IV (cardiac arrest after diagnosis of brain death, "controlled donor") or V (sudden cardiac arrest in patients in the intensive care unit, "uncontrolled donor") by the modified Maastricht classification of non-heart beating donors [15]. The preservation period averaged 13.2 ± 3.4 hours. In the vast majority of cases, only kidneys were taken from donors.

Selection of the donor-recipient pair was carried out considering the blood group and human leukocyte antigens of A, B and DRB1 loci. Depending on the number and combination of mismatches by the loci, the compatibility index was calculated [16]: the median was 6 (interquartile range from 6 to 7).

In all recipients, it was the first transplantation; there were no pre-existing anti-HLA antibodies. Antibody screening was performed by multiplex technology on the Luminex platform with LIFECODES Lifescreen Deluxe reagents (Immucor). Transplantation was performed only with negative cross-sectional tests (complement-dependent microlymphocytotoxic test).

All patients received standard induction therapy using anti-CD25 antibodies and methylprednisolone. A three-component immunosuppression protocol was used, tacrolimus (concentration control and subsequent dose adjustment), mycophenolates and prednisone in standard dosages [17].

All patients underwent standard postoperative clinical laboratory, radiological, and ultrasound examinations.

The control group included volunteers (N = 21) 55–70 years of age without acute inflammatory diseases and signs of renal failure. The average age in the control group was 61.1 ± 4.4, the average number of CIRS-G scores was 12.11 ± 6.04.

Immunophenotypy was performed by flow cytometry with the FACSCalibur apparatus (Becton Dickinson, USA). Multiparameter flow cytofluorimetric subpopulation analysis was made.

To assess the functional state of peripheral blood mononuclear cells, we used the method of vital computer laser cytomorphometry with the "Cytoscan" (MGIREA, Russia) laser phase-interference microscope. The method allows for capturing a phase-interference image of living cells and evaluate the level of anisotropy of nuclear chromatin, thus providing an indirect judgement of the degree of functional activity assessed by the functional activity index (FAI) of the core of the separated peripheral blood mononuclear cells.

The protocol of the present study was approved by the local ethics committee (protocol No. 4 of April 6, 2010) and by the decision of the academic council of the Vladimirsky Moscow Regional Research and Clinical Institute, Moscow (protocol No. 4 of April 19, 2010). All participants signed an informed consent.

STATISTICAL ANALYSIS

The distribution law compliance of the samples was checked by Shapiro–Wilk test. The variables with normal

distribution are presented as mean ± SD; those with the distribution differing from normal, as well as ordinal variables, are represented as the median and interquartile range: median (1st quartile; 3rd quartile).

To analyze the relationship between quantitative characteristics, Pearson correlation coefficient was used, the correlation coefficient (r), its 95% confidence interval (95% CI), and the determination coefficient (r^2) were calculated.

To analyze several samplings with a normal distribution, the analysis of variance with Post-hoc Tukey was implemented. The calculations were made with GraphPad Prism 8.0 (GraphPad Software, USA). 2-tailed significance was assessed. $p < 0.05$ was considered statistically significant.

RESULTS

To analyze the features of the cellular immunity unit activation at kidney transplantation, the differences in lymphocyte subpopulations in healthy volunteers and patients with CKD 5D stage receiving dialysis treatment before and after transplantation were analyzed (Fig. 1).

Comparing cells subpopulations in CKD 5D patients on dialysis with those of healthy volunteers, the variability is seen to significantly increase witnessing high heterogeneity of the dialysis patient population. With a statistically insignificant tendency to decrease in the proportion of CD3 cells ($p = 0.137$), the proportion of CD4 cells ($p = 0.009$) is significantly reduced and the proportion of CD8 cells ($p = 0.02$) increases. As a result of such multidirectional dynamics, the immunoregulatory index decreased (CD4/CD8 ratio), $p = 0.017$.

Besides, in dialysis patients, the number of natural killers increases ($p = 0.025$) and there is a tendency of the B cells ratio to slightly increase ($p = 0.135$), as well as increase in HLA-DR expression by CD3 cells ($p = 0.072$).

After KT, on day 5 after surgery, the proportion of CD3 cells statistically significantly increases ($p = 0.017$) mainly due to an increase in the proportion of CD4 cells ($p = 0.002$) with a slight increase in the proportion of CD8 cells ($p = 0.439$). As a result, the immunoregulatory index increased slightly ($p = 0.236$). The content of natural killers also increased ($p = 0.002$), and the proportion of B cells decreased slightly ($p = 0.277$). HLA-DR expression on CD3 cells increased significantly ($p < 0.0001$).

In CKD 5D patients, there is a decrease in the functional activity of peripheral blood mononuclear cells in comparison with healthy volunteers, which is reflected in a statistically significant ($p = 0.0138$) decrease in FAI. After KT, this indicator significantly increases compared with the indicator before KT ($p < 0.0001$) and exceeds the FAI value in healthy volunteers ($p < 0.0001$) (Fig. 2).

We noted interesting age-related features (Fig. 3).

In healthy volunteers, the functional activity of peripheral blood mononuclear cells gradually decreases

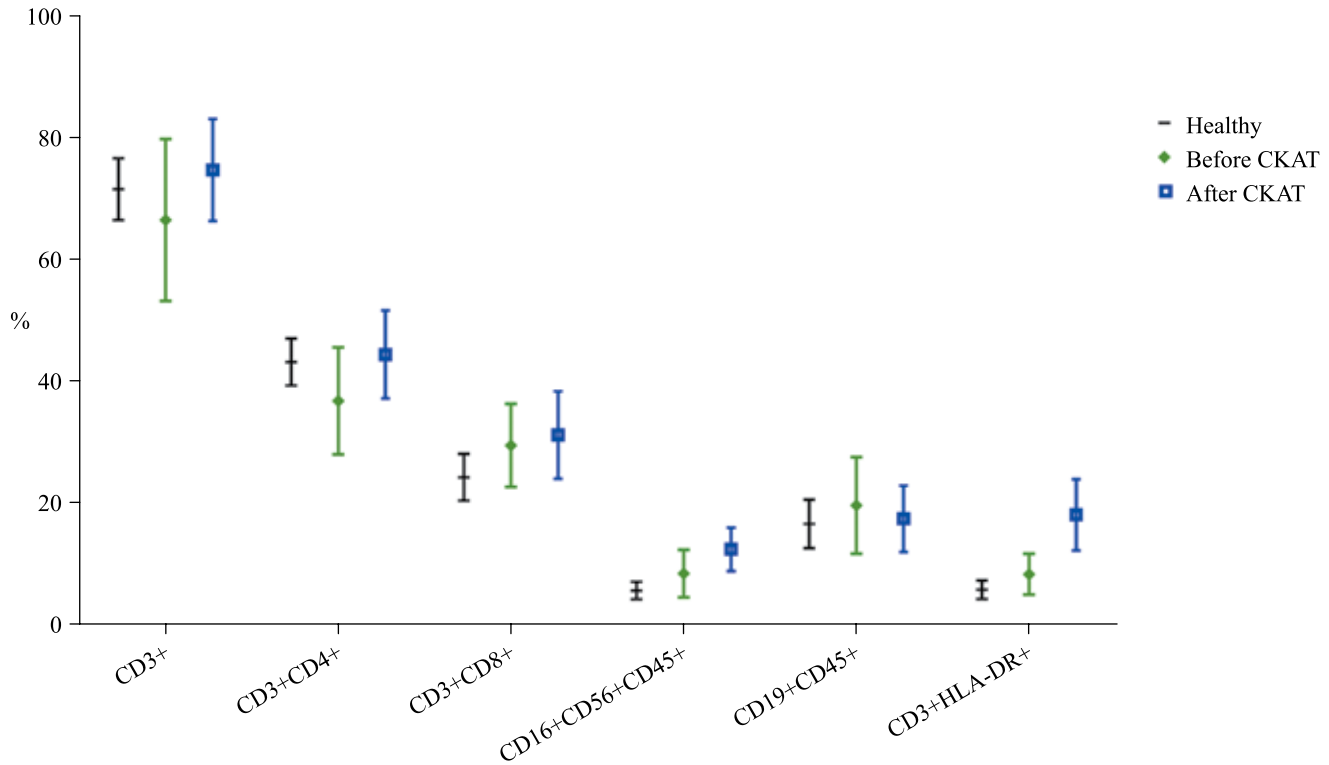

Fig. 1. Lymphocyte subsets, HLA-DR+ cell and natural killer cells fractions in healthy volunteers, CKD 5D patients before and after kidney transplantation

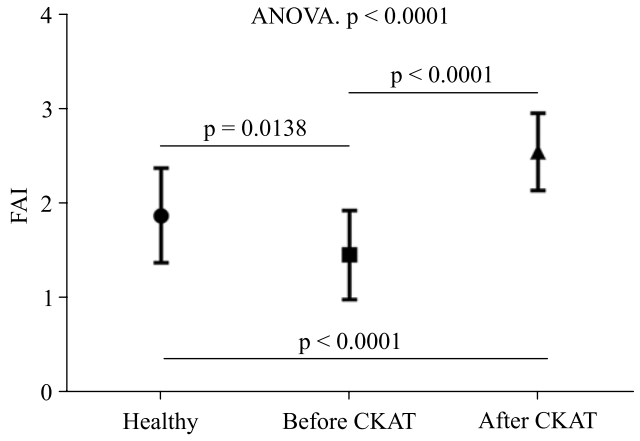

Fig. 2. Functional activity value of peripheral blood mononuclear cells in healthy volunteers, patients with CKD 5D before and after kidney transplantation

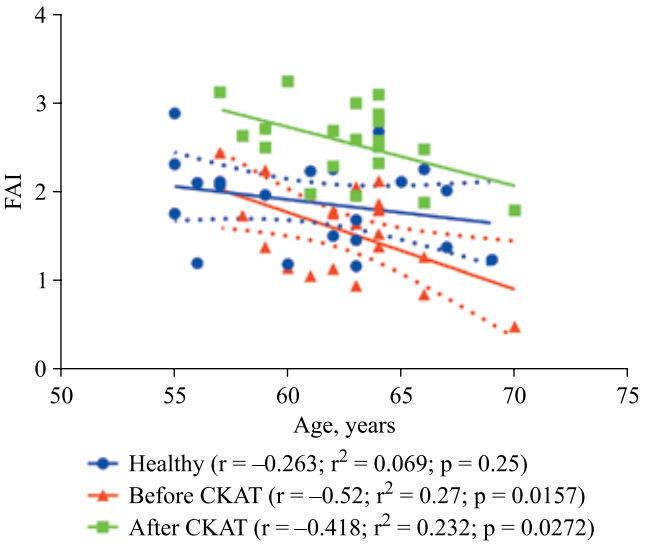

Fig. 3. Correlation of age and functional activity value of peripheral blood mononuclear cells in healthy volunteers, patients with CKD stage 5D before and after kidney transplantation

with age. Nevertheless, this relationship was weak (r = –0.263 [95% CI –0.6236; 0.1907], r^2 = 0.069) and did not reach the required level of statistical significance (p = 0.264). At the same time, in CKD5D patients, this dependence was of the same orientation, but was much stronger: (r = –0.52 [95% CI –0.7771; –0.1135], r^2 = 0.27) and was statistically significant (p = 0.0157). In patients after kidney transplantation, the average level of PFA was higher than before transplantation (p < 0.0001, Fig. 2), but the relationship with age was similar: r = –0.418 [95% CI –0.7559; –0.06256], r^2 = 0.175 and was statistically significant (p = 0.0272).

DISCUSSION

Age-related features of immune reactions have been known for long. The recipients of older age groups are featured by changes in the ratio of various subpopulations of lymphocytes, as well as in metabolism and functional potential of these cells [18–21]. We focused on the peculiarities of the subpopulation composition of lymphocytes and the morphofunctional state of peripheral blood mononuclear cells in CKD5D patients, namely in patients of an older age group before and after kidney transplantation.

The patients with chronic kidney disease are known to develop chronic inflammation combined with persistent native and adaptive immunity dysfunction [22]. The clinical manifestation of this fact, as well as its indirect confirmation, is an increased risk of infectious complications [23, 24] and malignant neoplasms [23, 25], as well as a decrease in the efficiency of vaccination [26, 27], as evidenced by the results of large studies.

We found that in stage 5 CKD patients, there is a decrease in the proportion of CD4 cells and an increase in the proportion of CD8 T cells, while their ratio is significantly reduced. Besides, there is a slight decrease in the total proportion of CD3 cells (statistically insignificant in the present study) with a significant increase in the variability of this indicator, which indirectly points to the heterogeneity of the CKD patient population.

There is evidence that the lymphopenia severity is associated with several factorsб the severity of impaired renal function and oxidative stress, the level of urea, creatinine, phosphorus, diabetes mellitus, etc. [28–32]. The change in the subpopulation composition of lymphocytes, as well as a decrease in their total number, is tied to an increased tendency towards apoptosis, a transformation of the cytokine profile, which is characteristic of patients with CKD [30, 33–35]. Besides, the dialysis procedure itself promotes apoptosis of T cells and reduces their proliferative ability [29, 31, 35].

Impaired T-cell regulation associated with progressive loss of renal function is often described as “premature aging of the immune system”, which is featured by a significant reduction in the native T-cell population and a relative increase in memory T-cells, a decrease in the ratio of CD4/CD8 T-cells, and an increased tendency to apoptosis, a change in the receptor repertoire of T cells and a reduction in telomere length. In patients with CKD of 25–45 years old, the indicators are comparable with those of healthy people of 60 to 80 [30, 36, 37].

The severity of changes in T-cell immunity is directly related to the results of kidney transplantation. An increase in the waiting time for kidney transplantation on dialysis promotes the accumulation of alloreactive T cells and is accompanied by an increased risk of acute transplant rejection [38, 39] and significantly worsens the results of transplantation [40–42]. Despite the fact that the characteristics of T-cell immunity influence the results of kidney transplantation [32, 43, 44], currently this knowledge does not allow modifying the existing

clinical practice by personalizing immunosuppressive therapy, which emphasizes the relevance of research in this direction. The fact that the features of violations of T-cell immunity (in the context of the "aging" of the immune system) can affect not only the immediate ones (since the existing disorders form a kind of pre-transplant background), but also the long-term results of kidney transplantation, according to research results, which have been shown that these disorders persist even after successful transplantation [32, 37].

Natural killer cells (NK) are one of the specialized subpopulations of lymphocytes that play an important role in antiviral and antitumor immunity, as well as in the regulation of homeostasis and inflammatory processes in tissues [45, 46]. In our study, the proportion of these cells in CKD patients was significantly greater than in healthy volunteers. This indicates the activation of native immunity, which may be of a compensatory-adaptive nature against the background of dysfunction of adaptive T-cell immunity in CKD [47]. On the other hand, an increase in the proportion of NK in patients with CKD can be explained by the active participation of these cells in the progression of CKD (regardless of the underlying etiology of kidney disease) [48–51].

The proportion of B cells in healthy individuals and CKD patients did not differ statistically significantly. Nevertheless, in the latter it was slightly higher, as was the heterogeneity of this indicator. There are also several non-mutually exclusive explanations for this. B cells play an important role in the pathogenesis of many autoimmune renal lesions [52–54]. In our study, a significant proportion of patients with formal CKD were glomerulonephritis and hypertension (10 of 21). Nevertheless, it should be borne in mind that histological verification of the diagnosis (causes of CKD) is extremely rare, which does not exclude the possibility of another reason. The fact that the structure of the causes of CKD in our country, according to the data of the All-Russian Register [2], is significantly different from other large registers [1, 3] also indirectly testifies to this. In addition, for example, we did not take into account the form of glomerulonephritis (which can be attributed to the limitation of the study). Another explanation (arising from the first) may be the use of various drugs to treat the underlying disease (causes of CKD). For example, rituximab (an anti-CD20 monoclonal antibody) is widely used to treat many autoimmune diseases: ANCA-associated vasculitis, membranous nephropathy, lupus nephritis, mixed cryoglobulinemia, nephrotic syndrome with minimal changes, focal segmental glomerulosclerosis etc. [47, 55]. It should be noted that there is no consensus on the polarity of the change in the proportion of B cells in CKD patients [56, 57].

In the present study, CKD patients had an increased level of HLA-DR expression compared with healthy individuals. This may be due to chronic inflammation of a low degree of activity characteristic of CKD patients [22, 58, 59].

In kidney transplant recipients, on day 5 after transplantation, an increase in the proportion of CD3 cells, CD4 cells, NK, as well as the expression of HLA-DR was noted. This can be considered, on the one hand, as a nonspecific response to surgery [60–63]; on the other hand, a synchronous increase in the proportion of CD4 cells and NK may be a specific reaction to allotransplantation [64–69].

If we do not take into account the problem of presensitized patients, then severe humoral reactions to the graft, the clinical manifestation of which is the rejection reaction, usually refer to the late postoperative period and occur with the active participation of adaptive immunity. We deliberately sampled patients without preexisting anti-HLA antibodies. In the pathogenesis of ischemia-reperfusion syndrome, the main role is played by nonspecific reactions of native immunity with activation, mainly of the cellular link. In this regard, we evaluated the morphological and functional parameters of the cellular immunity unit (as the main effector unit) in renal allograft recipients.

After transplantation, an increase in the activity of peripheral blood mononuclear cells occurs (even compared with healthy volunteers). This is quite expected and can be explained by both nonspecific and specific mechanisms. At the same time, in CKD 5patients, both before and after transplantation, a statistically significant relationship between PFA and age was revealed, which was not observed in the group of healthy volunteers. This fits quite well into the modern concept of violations of the morphofunctional state of the cells of the immune system formed against the background of CKD. However, the revealed dependence testifies in favor of the fact that the age of the recipient is an important factor that can influence the choice of immunosuppressive therapy regimen. This becomes even more relevant in view of the fact that elderly patients are characterized by a significant change in the metabolism of immunosuppressive drugs and, accordingly, their concentration in the blood (in particular, calcineurin inhibitors), which makes it even more difficult to achieve a shaky balance between insufficient and excessive immunosuppression [8–11, 70, 71].

CONCLUSION

Older CKD patients before and after CKAT are susceptible to a significant change in the morphofunctional state of peripheral blood mononuclear cells and the subpopulation of lymphocytes. Moreover, the severity of changes in the functional state of these cells has a strong relationship with age, which is not observed in the group of healthy volunteers. This should be considered

when choosing immunosuppressive therapy in kidney transplant recipients of an older age group.

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The authors declare no conflict of interest.

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