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Characteristics of B-lymphocyte subpopulations in renal transplant recipients

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Abstract

Introduction. *The presence of multiple subsets of B-cells with specific regulatory functions capable of modulating inflammatory responses has been detected. Most of the studies of B_{regs} function were carried out in the context of autoimmune and infectious diseases, whereas the objective of this research was to study the characteristics of the main, activated and tolerogenic subpopulations of B lymphocytes in patients who underwent kidney transplantation.*

Objectives. *To study the indices of B-lymphocyte subpopulations and determine their role in the development of immunological tolerance after kidney transplantation.*

Material and methods. *We have examined 197 recipients who underwent kidney transplantation. We determined B-lymphocyte subpopulation levels ($CD19^+IgD^+CD27^+$ and $CD19^+IgD^-CD27^+$) before transplantation, on the 1st, 3rd, 7th and 30th days after the transplantation. Allograft function was assessed on day 7 with the*

division of patients into two groups: with primary graft function and graft dysfunction.

Results and discussion. *Significant differences were revealed between the groups of recipients over three months in the following cell subpopulation levels $CD19^+IgD^+CD27^+$ and $CD19^+IgD^-CD27^+$. During the first 7 days, lower levels of these subpopulations were associated with satisfactory allograft function. However, by the 90th day after surgery, an increase in $CD19^+IgD^+CD27^+$ B lymphocytes was noted in the group of patients with graft dysfunction.*

Conclusions. *Low levels of not-switched ($CD19^+IgD^+CD27^+$) and switched ($CD19^+IgD^-CD27^+$) memory B lymphocytes in the peripheral blood of kidney transplant recipients are associated with a favorable postoperative course. We have found that on the 3-rd post-transplant day, the relative level of non-switched memory B lymphocytes ($CD19^+IgD^+CD27^+$) exceeding or equal to 11.47%, and the level of switched memory B lymphocytes ($CD19^+IgD^-CD27^+$) exceeding or equal to 20.74% might predict the development of early renal graft dysfunction with a sensitivity and specificity of 88.40% and 84.30% for the former parameter and of 88.70% and 82.40% for the latter one, respectively.*

Keywords: kidney transplantation, $CD19^+IgD^+CD27^+$ and $CD19^+IgD^-CD27^+$ B lymphocytes, renal graft dysfunction

Conflict of interests Authors declare no conflict of interest

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CG, comparison group

CI, confidence interval

GDF, graft dysfunction

HD, hemodialysis

IST, immunosuppressive therapy

PD, peritoneal dialysis

PGF, primary graft function

PS, pre-dialysis stage

Introduction

The immune system includes a complex interrelation between many different cell types. Over the past decade, T cells, dendritic cells, and macrophages have been identified as key cells that regulate the immune response, linking the innate and adaptive immunity. Many research groups, when assessing various immunological parameters responsible for the development of tolerance, relied mainly on the parameters of T-cell activity, and specifically, T-regulatory lymphocytes [1]. At the same time, B-lymphocytes, traditionally known for their role in the production of antibodies, the antigen presentation, and the production of the cytokines dictated by T-cells, have recently acquired a different significance in the development of the immunological response [2]. Numerous subsets of B cells with specific regulatory functions capable of modulating T-cell and chronic inflammatory responses have been found to exist [3].

During maturation, the set of B cell markers changes in response to antigen activation and further proliferation. The main membrane antigen CD19 is used to verify peripheral B lymphocytes. According to the degree of expression of other surface markers (IgD, IgM, CD27, CD38, CD10), several subpopulations of B cells are distinguished. Thus, the

following types of mature B lymphocytes are distinguished: proper B cells ($CD19^+IgD^+CD27^-$), naive B lymphocytes) – inactivated B lymphocytes that did not come into contact with the antigen. They have a weak affinity for many antigens. Memory B cells ($CD19^+IgD^+CD27^+$, non-switched memory B lymphocytes) are activated B lymphocytes [4]. As B cells mature, they switch from the synthesis of IgM and IgD to the synthesis of IgG, IgA, and IgE by memory B cells (the $CD19^+IgD^-CD27^+$, switched memory B lymphocytes, main antibody-producing cells). The final stage of differentiation of activated B cells includes plasma cells, which, unlike other B cells, have a small number of membrane antibodies and are able to secrete antibodies.

Recent studies have highlighted the existence of B cells with regulatory properties that have been termed B_{regs}, similar to regulatory T cells (T_{regs}). Despite the fact that there is little information in the literature about the role of this subpopulation in the development of transplant tolerance, some researchers note a link between the B_{regs} level and kidney graft survival [5]. Thus, the activation of B cells in autoimmune diseases is associated with impaired homeostasis of peripheral blood B lymphocytes and pronounced expansion of CD27+ memory B cells [6, 7]. Most studies on the function of B_{regs} were conducted in the context of autoimmune and infectious pathology, while the aim of the present study was to investigate the parameters of the main, activated and tolerogenic B-lymphocyte subpopulations in patients who underwent kidney transplantation.

In previous studies, we have identified the relationship and predicative significance of determining the level of naive $CD19^+IgD^+CD27^-$ B lymphocytes in predicting the initial function of a kidney graft in the early postoperative period based on a scoring system using the categorical regression procedure [8]. We continued to study the

dynamics of the $CD19^{+}IgD^{+}CD27^{+}$ non-switched, and $CD19^{+}IgD^{-}CD27^{+}$ switched, memory B-cell subpopulations and their possible prognostic significance in renal allotransplantation.

The study purpose was to investigate the parameters of B-lymphocyte subpopulations and determine their role in the development of immunological tolerance after kidney transplantation.

Material and methods

We studied 197 patients who underwent kidney transplantation in the Transplantation, Endocrine and Reconstructive Surgery Department of the Republican Research Center for Radiation Medicine and Human Ecology. The study met the criteria of Helsinki Declaration of 1975, was approved by the Ethics Committee of the Republican Research Center for Radiation Medicine and Human Ecology (Protocol No. 5 of 02.12.2013).

The criteria for inclusion in the study group were: primary renal transplantation; induction therapy with monoclonal anti-CD25 antibodies; three-component immunosuppressive therapy. A negative result of a direct cross-match test was observed in 100% of cases.

Patients were divided into two groups according to the type of the kidney graft functioning: patients with primary graft function (PGF, n=101) (mean age 45.46±1.28 [42.92; 47.99] years) and patients with early graft dysfunction (GDF, n=96) (46.26±1.20 [43.87; 48.65] years) (p=0.772). Early renal graft function was assessed on day 7 after surgery based on blood creatinine levels and the need for dialysis. If creatinine values were below 300 mmol/L, the function was considered primary (PGF), if values were equal to or exceeding 300 mmol/L, and if dialysis was required in the first week after transplantation, patients were assigned to the GDF group [9]. Ninety healthy volunteers participated as a comparison group (CG).

The average duration of dialysis was 30.31±2.79 months [95% CI 24.77; 35.86] in the PGF group, and 37.40±3.71 months [95% CI 30.03; 44.77] in the GDF group. As for the dialysis duration, there was the following patient distribution: 13 patients (12.87%) in the PGF group, and 20 (from 20.83%) in the GDF group had been on dialysis for 5 years and longer; 62 patients (61.39%) in the PGF group, and 54 (56.25%) in the GDF group had been on dialysis for 1 year to 5 years; 24 patients (23.76%) in the PGF group, and 21 (21,88%) patients in the GDF group had been on dialysis for up to 1 year. There were 2 patients (1.98%) in the PGF group, and 1 patient (1.04%) in the GDF group who were at pre-dialysis stage (PS).

The compared PGD and GDF groups had no statistically significant differences in patient gender (PGD: 38 (37.62%) women, 63 (62.38%) men vs. GDF: 37 (38.54%) women, 59 (61.46%) men, p=506), the type of dialysis performed before transplantation (PGF: hemodialysis (HD) in 76 (75.25%) patients, peritoneal dialysis (PD) in 23 (22.77%), PS in 2 (1.98%) patients vs. DFT: HD in 81 (84.38%) patients, PD in 14 (14.58%), PS in 1 (1.04%), p=0.145), cold ischemia time of the donor organ (PGF: 11.93±0.4 hours [11.11; 12.75] vs. GDF: 12.79±0.46 hours [11.87; 13.70], p=0.274).

All patients received immunosuppressive therapy according to the clinical protocols of kidney transplantation (Appendix 1 to the Order No. 6 of 05.01.2010 issued by the Ministry of Health of the Republic of Belarus).

The flow cytometry with multiple translational gating was used to determine the immunological characteristics of kidney transplant recipients.

Immunological examination of patients was performed before surgery, on day 1st, 3rd, 7th, and 30th after surgery.

Method for determining the relative and absolute number of B-lymphocyte subpopulations

Blood was collected from the ulnar vein into test tubes containing an anticoagulant (ethylenediaminetetraacetic acid). We used monoclonal antibodies labeled with fluorochromes to surface antigens IgD FITC, CD27 PC5.5, CD19 APC (Beckman Coulter and BD, USA) according to the manufacturer's instructions. Blood with labeled antibodies for flow cytometry was incubated for 15 minutes in the dark. Opti Lyse B was used for lysis of erythrocytes. Samples were analyzed on a FACS Canto II flow cytofluorometer (BD, USA). At least 20,000 events were accumulated. Gating was performed for 19^{+}cells. Non-switched and switched memory B cells had the $CD19^{+}IgD^{+}CD27^{+}$ and $CD19^{+}IgD^{-}CD27^{+}$ immunophenotypes, respectively. The absolute value of all B-lymphocyte subpopulations in peripheral blood was obtained by mathematical calculation.

Statistical processing of the results was performed using the Statistica 10.0 software package. Descriptive statistics of qualitative characteristics are presented in absolute and relative frequencies, while quantitative characteristics are presented in the following format: mean (confidence interval) - M [Confidence -95%; +95%], and median (interquartile range) - Me [Q25; Q75]. To compare the values, we used the numerical characteristics method (Mann-Whitney U Test, Wilcoxon Matched Pairs Test) with an estimate of the distribution of variables. For nominal variables, conjugated tables were analyzed and frequency differences were estimated using the Pearson Chi-square test and the two-tailed Fisher's exact test. The results were considered statistically significant when p-value was at level lower than 0.05.

Results

After studying the dynamics of $CD19^+$ lymphocytes in the groups of kidney transplant recipients during the first month and comparing them with the values of the comparison group, we noted the following peculiarities. Before kidney transplantation, the relative level of $CD19^+$ lymphocytes was equal to 9.20% [5.30;10.60] in the PGF group and 8.50% [7.10;11.80] in the GDF group, which was lower than the values in the comparison group (11.00% [8.10;13.90]) ($p_{0PGF/CGrel}$=0.019, $p_{0GDF/\ CGrel}$ =0.014).

The absolute content of $CD19^+$ lymphocytes was also lower in PGF (0.12×10^9cells/L [0.08; 0.18]) and GDF (0.14×10^9cells/L [0.09; 0.21]) groups than in the comparison group (0.23×10^9cells/L [0.15; 0.33]) ($p_{0PGF/CGabs}$ <0.0001 and $p_{0GDF/CGabs}$ <0.0001).

There were no statistically significant differences in the relative and absolute content of $CD19^+$ lymphocytes between the PGF and GDF groups ($p_{0PGF/GDFrel}$=0.575, $p_{0PGF/GDFabs}$=0.964).

From the first day after transplantation, there was a statistically significant increase in the relative number of B lymphocytes in all groups ($p_{0,1\ PGF}$<0.0001, $p_{0,1\ GDF}$<0.0001), meanwhile, the level of B-lymphocytes both in the PFT and DFT groups was significantly higher than in the CG ($p_{PGF1/CGrel}$=0.018, $p_{GDF1/CGrel}$=0.012). The absolute number of $CD19^+$ in the PGF and GDF groups ($p_{1PGF/CGabs}$<0.0001, $p_{1GDF/CGabs}$<0.0001, $p_{3PGF/CGabs}$=0.036, $p_{3GDF/CGabs}$) kept below the level in the CG for up to 3 days.

By day 3, there was a progressive increase in the relative number of B lymphocytes; in the PFT and DFT groups, the level of this subpopulation increased 2-fold and amounted to 19.40% [15.20; 24.40] and 17.45% [13.25; 26.30], respectively ($p_{3PGF/GDFrel}$=0.386).

A relative level of B lymphocytes on 7 post-transplantation day decreased and made 12.20% [7.50; 18.00] in the PGF group and 13.30% [9.60; 19.60] in the GDF group; there were no statistically significant differences between the groups in this parameter ($p_{7PGF/GDFrel}$=0.277, $R_{7PGF/GDFabs}$=0.269).

Further monitoring of the relative number of B lymphocytes on day 30 did not reveal such considerable dynamics. In the PGF group, the $CD19^+$ lymphocyte level did not significantly differ from the level in the GDF group and made 13.80% [11.10; 19.10], while the value in the GDF group was 16.20% [9.50; 23.30] ($p_{30PGF/GDFrel}$=0.240). The absolute level of B lymphocytes in the PGF group made 0.29×10^9cells/L [0.14; 0.45]; and in the group of DFT, the content of $CD19^+$ lymphocytes was 0.20×10^9cells/L [0.14; 0.41], which was comparable to the level in the PGF group ($p_{30PGF/GDFabs}$=0.197).

The content of the minor $CD19^+IgD^+CD2727^+$ B-lymphocyte subpopulation in the early post-transplant period is shown in Table. 1.

Table 1. The level of $CD19^+IgD^+CD27^+$ in the peripheral blood of patients of the compared groups (Me [LQ; UQ])

Day	Measuring units	CG	PGF	GDF
0	%	14.40 [8.90;19;90] % 0.11 [0.08;0.17]10^9 cells/L	9.86*# [9.70;11.13]	23.44 [13.61;36.07]
	10^9 cells/L		0.01*# [0.005;0.018]	0.04 [0.02;0.06]
1	%		7.52*# [6.00;8.63]	30.05 [17.41;37.50]
	10^9 cells/L		0.01*# [0.004;0.013]	0.02 [0.02;0.04]
3	%		10.19*# [9.39;11.42]	21.63 [14.61;31.45]
	10^9 cells/L		0.02*# [0.11;0.026]	0.01 [0.01;0.02]
7	%		10.33# [9.15;12.35]	25.34 [14.11;36.57]
	10^9 cells/L		0.02# [0.018;0.025]	0.04 [0.02;0.06]
30	%		12.61# [11.24;14.60]	12.01 [10.82;13.21]
	10^9 cells/L		0.02 [0.013;0.054]	0.02 [0.02;0.04]

Notes: * p <0.05 relative to the data of the CG;

# p <0.05 relative to the data of the GDF group.

Interestingly, before surgery, the relative and absolute levels of $CD19^{+}IgD^{+}CD27^{+}$ in the PGF group were significantly lower than in the comparison group and the GDF group ($p_{0PGF/GDF}<0.0001$, $p_{0PGF/CG}=0.002$, $p_{0GDF/CG}=0.039$). In the early post-transplant period, the dynamics of the number of non-switched $CD19^{+}IgD^{+}CD27^{+}$ memory B lymphocytes in the GDF group differed from the dynamics in the PGF group. Thus, in the PGF group, an increase in the relative number of $CD19^{+}IgD^{+}CD27^{+}$ lymphocytes was observed, but a statistically significant predominance remained in the GDF group ($p_{3PGF/GDF}<0.0001$, $p_{7\ PGF/GDF}<0.0001$) (Fig. 1).

On day 30 of follow-up, the level of non-switched memory B lymphocytes in the GDF group decreased by 2 times, while their content became lower than in the PGF group (Mann-Whitney U Test:$_{30PGF/GDFrel}=0.022$) (see Fig. 1).

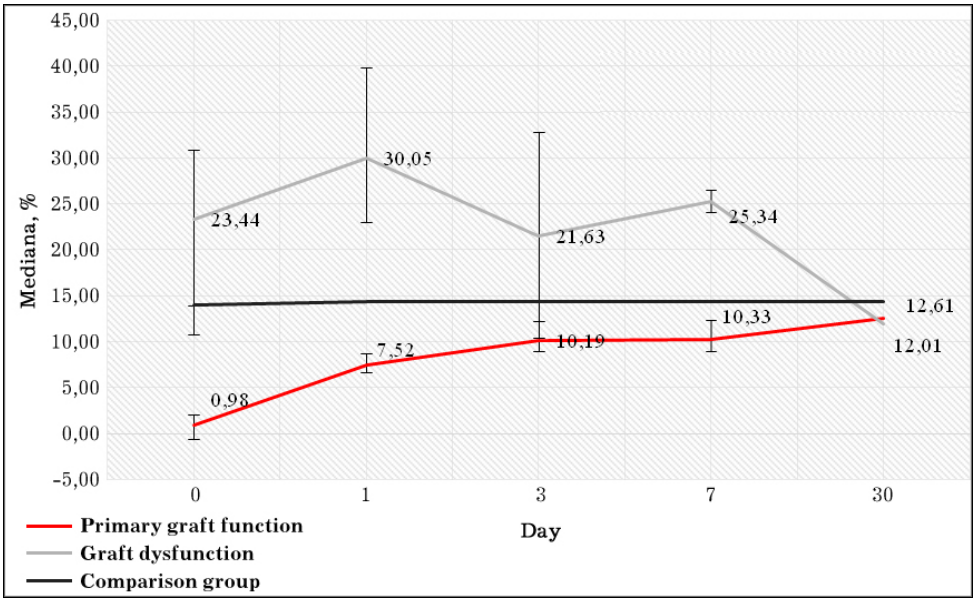

Fig. 1. Dynamics of the relative level of $CD19^{+}IgD^{+}CD27^{+}$ lymphocytes in renal allograft recipients

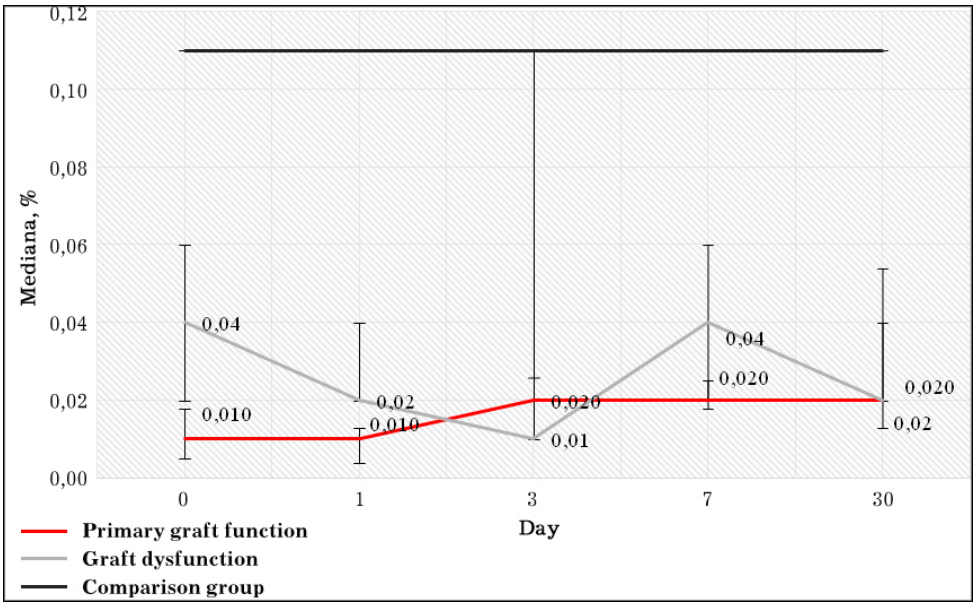

Fig. 2. Dynamics of the absolute level of $CD19^+IgD^+CD27^+$ lymphocytes in renal allograft recipients

The content of the minor $CD19^+IgD^-CD27^+$ subpopulation of switched memory B lymphocytes in the early post-transplant period is presented in Table 2.

Table 2. The level of $CD19^+IgD^-CD27^+$ in the peripheral blood of patients of the compared groups (Me [LQ; UQ])

Day	Measuring units	CG	PGF	GDF
0	%	22.80 [19.10;27.70] % 0.03 [0.02;0.06]10^9 cells/L	19.00$^{\#}$ [14.20;25.85]	13.69 [11.60;14.79]
	10^9 cells/L		0.02$^{*\#}$ [0.018;0.034]	0.017 [0.01;0.024]
1	%		16.54$^{*\#}$ [11.45;20.76]	8.86 [6.65;10.41]
	10^9 cells/L		0.02$^{*\#}$ [0.007;0.029]	0.010 [0.006;0.016]
3	%		15.28$^{*\#}$ [12.11;18.44]	31.68 [29.24;33.23]
	10^9 cells/L		0.02* [0.015;0.046]	0.029 [0.017;0.067]
7	%		12.26$^{*\#}$ [10.76;15.40]	28.03 [18.95;38.33]
	10^9 cells/L		0.02$^{*\#}$ [0.019;0.034]	0.038 [0.029;0.083]
30	%		15.85* [9.81;21.48]	13.97 [12.30;16.43]
	10^9 cells/L		0.03 [0.014;0.062]	0.032 [0.016;0.052]

Notes: * p <0.05 relative to the data of the CG;

# p <0.05 relative to the data of the GDF group.

During the period of monitoring the relative and absolute numbers of $CD19^{+}IgD^{-}CD2727^{+}$ switched memory B-lymphocytes, the following specific features were revealed (Figs. 3, 4).

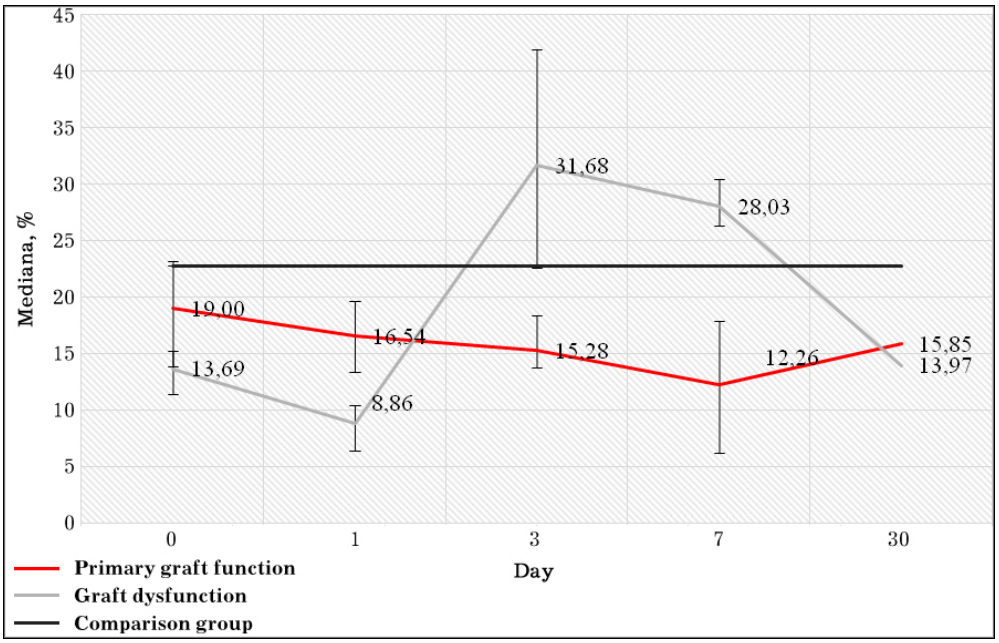

Fig. 3. Dynamics of the relative level of $CD19^{+}IgD^{-}CD27^{+}$ lymphocytes in renal allograft recipients

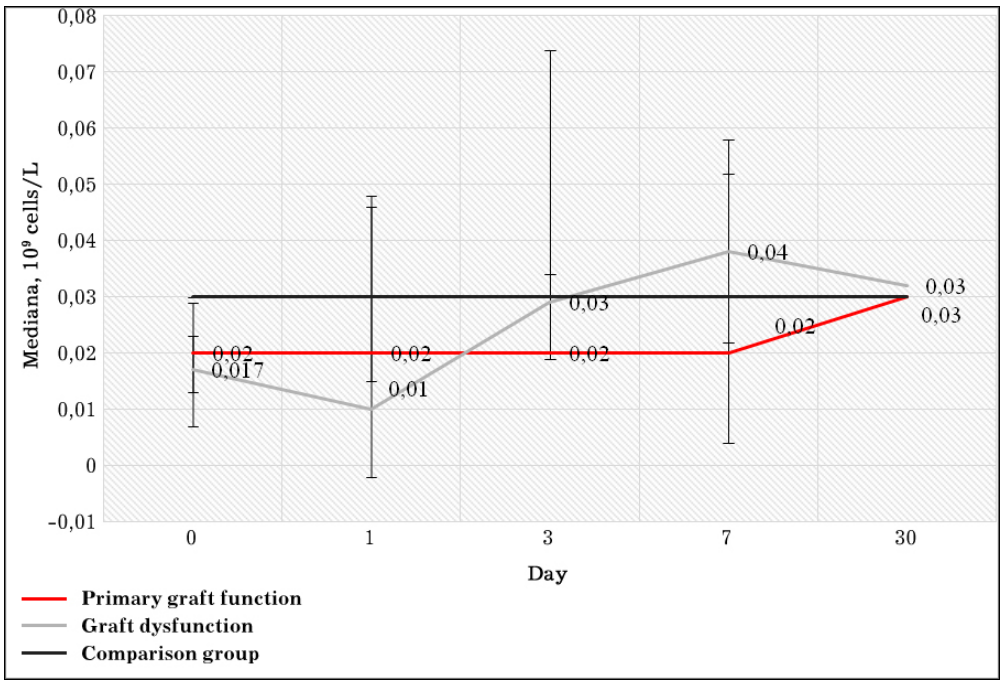

Fig. 4. Dynamics of the absolute level of $CD19^{+}IgD^{-}CD27^{+}$ lymphocytes in renal allograft recipients

Before transplantation, the relative level of $CD19^{+}IgD^{-}CD27^{+}$ lymphocytes in the GDF group was significantly lower than in the PGF group and the comparison group (Mann–Whitney U Test $p_{0PGF/GDFrel}<0.0001$, $R_{0PGF/CGrel}=0.120$, $p_{0GDF/CGrel}<0.0001$) (Fig. 3). The absolute number of switched memory B cells before surgery was higher in the PGF group than in the comparison group; and in the GDF group it was lower than in the CG (Mann–Whitney U Test $p_{0PGF/CGabs}=0.120$, $p_{0GDF/CGabs}<0.0001$) (Fig. 4). Comparison between groups showed that the level of $CD19^{+}IgD^{-}CD27^{+}$ lymphocytes in the GDF group was statistically significantly lower (Mann–Whitney U Test $p_{0PGF/GDFabs}<0.0001$).

In the PGF group, the relative number of switched memory B lymphocytes had gradually decreased by 21.03% by day 7 in response to immunosuppressive therapy and was statistically significantly lower than in the GDF group, as well as lower than in the CG (Mann-Whitney U Test $p_{7PGF/GDFrel}<0.0001$).

At day 30 of immunological monitoring, the content of $CD19^{+}IgD^{-}CD27^{+}$ lymphocytes in the PGF group remained without significant dynamics; and in the GDF group, the level of switched memory B cells decreased 2-fold and was significantly below normal (Mann–Whitney U Test $p_{30PGF/GDFrel}<0.0001$, $p_{30PGF/CGrel}=0.002$, $p_{30GDF/CGrel}<0.0001$).

In order to establish diagnostic characteristics for predicting the development of renal graft dysfunction based on the levels of $CD19^{+}IgD^{+}CD27^{+}$ and $CD19^{+}IgD^{-}CD27^{+}$ B lymphocytes on the 3rd post-transplant day using ROC analysis, the diagnostic potential of these parameters were identified (Table 3).

Table 3. Diagnostic characteristics of the levels of $CD19^{+}IgD^{+}CD27^{+}$ and $CD19^{+}IgD^{-}CD27^{+}$ in predicting the development of renal graft dysfunction

Parameter	Area under the curve	Cut-off point	Sensitivity	Specificity	Asymptomatic 95% CI	
					Lower limit	Upper limit
$CD19^{+}IgD^{+}CD27^{+}$, %	0.903 p<0.001	≥11.77	0.884	0.843	0.845	0.960
$CD19^{+}IgD^{-}CD27^{+}$, %	0.887 p<0.001	≥20.74	0.887	0.824	0.825	0.933

As follows from the data in Table 3, the relative level of CD19+IgD+CD27+ B cells exceeding or equal to the value of 11.77% and the level of CD19+IgD-CD27+ B cells exceeding or equal to the value of 20.74% on the 3rd post-transplant day, allow predicting the development of early renal graft dysfunction (with sensitivity of 88.40%, specificity 84.30%, and with sensitivity of 88.70%, specificity of 82.40%, respectively) (Fig. 5, 6).)

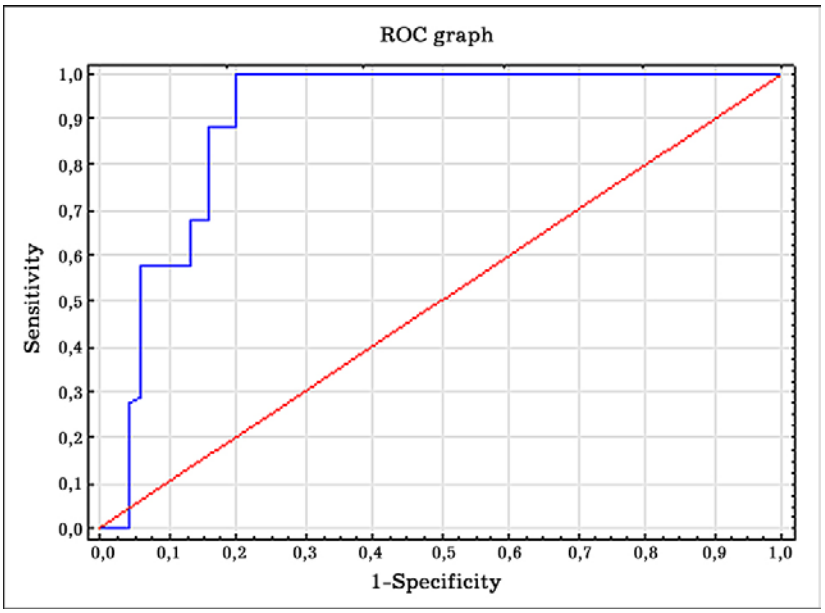

Fig. 5. Diagnostic characteristics of the relative level of $CD19^{+}IgD^{+}CD27^{+}$ B-lymphocytes in predicting renal allograft dysfunction

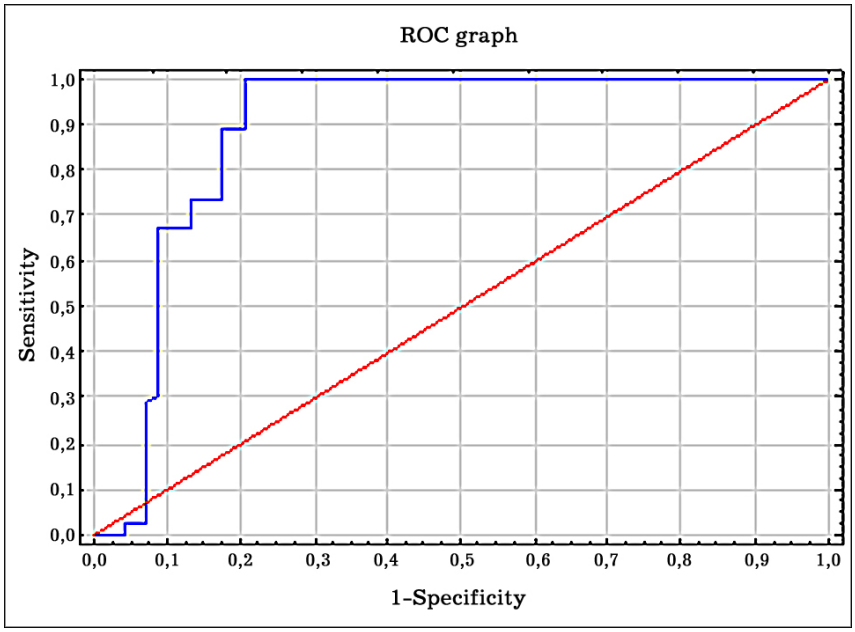

Fig. 6. Diagnostic characteristics of the relative level of $CD19^+IgD^-CD27^+$ B-lymphocytes in predicting renal allograft dysfunction

The ROC analysis of the diagnostic potential of the absolute levels of $CD19^+IgD^+CD27^+$ B lymphocytes (area under the curve 0.442; p=0.340) and $CD19^+IgD^-CD27^+$ (area under the curve 0.472; p=0.645) memory B-lymphocytes showed their low predictive value.

Discussion

As noted earlier, recipients with early renal graft dysfunction were characterized by a low level of both relative and absolute values of naive ($CD19^+IgD^+CD27^-$) B lymphocytes, which is consistent with the data of some authors indicating a decrease in the relative number of naive B-lymphocytes in the peripheral blood in some autoimmune diseases [8, 10-13]. In addition, we found good predictive characteristics of elevated levels of $CD19^+IgD^+CD27^+$ B lymphocytes and $CD19^+IgD^-CD27^+$ memory B lymphocytes on the 3rd post-transplant day in predicting the development of renal graft dysfunction.

According to our data, if the levels of non-switched $CD19^+IgD^+CD27^+$ memory B lymphocytes showed a statistically

significant predominance in the GDF group from the first day and during the first 7 days, then in the level of switched $CD19^{+}IgD^{-}CD27^{+}$ memory B lymphocytes, the similar changes were detected only from the 3rd post-transplant day. Thus, an increase in the relative level of the memory B lymphocyte subpopulation on day 3 after surgery allows predicting the development of early renal graft dysfunction. These changes in B-lymphocyte homeostasis in peripheral blood reflect a loss of immunological tolerance, which was indicated by some researchers [14, 15]. In addition, the working groups studying immunological tolerance, such as the Immune Tolerance Network (ITN) and the EU Indices of Tolerance, have also reported the role of B lymphocytes in tolerance development. Their study showed that immunologically resistant patients have an increased number of naive B cells [16].

In his studies, T. Matsushita revealed that early inhibition of naive and switched B lymphocytes contributed to a more severe course of autoimmune pathology, namely, of experimental autoimmune meningoencephalitis. In the experimental group, an early manifestation of the disease in mice was observed with more pronounced paresis of the limbs, and 14% of animals required euthanasia, while in the control group all animals remained alive [17].

Thus, the B-lymphocyte population plays an important role in the formation of immunological tolerance; and further studying the levels of B-lymphocyte subpopulations, clinical and laboratory parallels in kidney transplant recipients in the late postoperative period is important in developing new approaches to immunological monitoring of this group of patients.

Conclusions

1. A prognostically favorable dynamics of switched ($CD19^+IgD^-CD27^+$) lymphocytes is a decrease of their relative blood level from 16.54% [11.45; 20.76] to 15.85% [9, 81; 21.48] ($p_{1,30PGF}$ = 0.047) in response to ongoing immunosuppressive therapy, a noticeable increase in the blood level of non-switched CD19+IgD+CD27+ memory B lymphocytes from 7.52% [6.00; 8.63] to 12.61% [11.24; 14.60] ($p_{1,30PGF}$ <0.0001) from the 1st to the 30th post-transplant days.

2. The immunological peculiarities in renal graft dysfunction are characterized by a significant increase in the relative content of $CD19^+IgD^-CD27^+$ lymphocytes in blood from 8.86% [6.65; 10.41] to 13.97% [12.30; 16.43] ($p_{1,30GDF}$ <0.0001), a significant decrease in the content of $CD19^+IgD^+CD27^+$ B lymphocytes from 30.05% [17.41; 37.50] to 12.01% [10.82; 13.21] ($p_{1,30GDF}$ <0.0001) from the 1st to the 30th post-transplant days.

3. The level of non-switched $CD19^+IgD^+$CD27+ B memory lymphocytes exceeding or equal to 11.47% might predict the development of early renal graft dysfunction with the sensitivity of 88.40% and specificity of 84.30%.

4. The level of switched $CD19^+IgD^-CD27^+$ memory B lymphocytes exceeding or equal to 20.74% might predict the development of early renal graft dysfunction with the sensitivity of 88.70% and specificity of 82.40%.

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