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Studying some lymphocyte subpopulations in search for predictors of renal graft dysfunction

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Introduction. *One of the main problems in transplantology is the detection of simple, reliable and non-invasive markers that could predict adverse immune reactions and adjust immune suppressive therapy in allograft recipients in a timely manner.*

Objective. *To determine the immunological criteria for the prediction of a graft dysfunction.*

Material and Methods. *We have examined 197 recipients who underwent kidney transplantation. All of them were immunologically examined with the identification of more than 40 subpopulations of leukocytes. Allograft function was assessed on day 7 with the division of patients into two groups: with either primary or graft dysfunction. Simple and multiple logistic regressions were used to predict a graft dysfunction. Preliminary statistical analysis was performed using nonparametric statistics.*

Results and Discussion. *A scoring system to predict the graft function has been worked out. At $CD19^+IgD^+CD27^-$≤72.7%, score 1 is assigned, and 0 score is given at > 72.7%. At $CD3^+CD8^+CD69^+$>9.7% score 1 is assigned, and 0 score is given at $CD3^+CD8^+CD69^+$≤9.7%. Total score is calculated by summing up the scores. The total score = 0 predicts a primary graft function; total score ≥1 predicts a graft dysfunction. This scoring system has the sensitivity of 91.9%, the specificity of 100%, the accuracy of 94.9%, positive predictive value of 1 and negative predictive value of 0.877.*

Conclusions. *1. Percentage of $CD19^+IgD^+CD27^-$ and $CD3^+CD8^+CD69^+$ subpopulations can be used to predict a graft dysfunction. 2. At values of $CD19^+IgD^+CD27^-$ not exceeding 72.7% and $CD3^+CD8^+CD69^+$ more than 9.7%, the development of a graft dysfunction can be anticipated.*

Keywords: kidney transplantation, $CD19^+IgD^+CD27^-$, $CD3^+CD8^+CD69^+$, delayed graft function

CI, confidence interval

GD, graft dysfunction

HD, hemodialysis

IST, immunosuppressive therapy

KT, kidney transplantation

NPV negative predictive value (*of a test result*)

PD, peritoneal dialysis

PGF, primary graft function

PPV positive predictive value (*of a test result*)

PtD, prior to dialysis

R(A)G, renal (allo)graft

TS, total score

Introduction

Kidney transplantation is the most effective treatment for patients with end-stage renal disease. According to a number of authors, graft dysfunction (DFT) is one of the main factors affecting a 1-year graft survival, the length of hospital stay, and the incidence of acute rejection episodes [1, 2].

According to the literature, GD is observed in 20–33% of recipients of renal allografts obtained from a deceased donor and in 3–5% of live donor renal transplant recipients [2–4]. In transplantation, models have been created for calculating the risk of developing donor organ dysfunction based on such parameters as the cold ischemia time, allograft quality, donor creatinine level, donor body mass index, patient's age and concomitant diseases, immunological sensitization [3, 5, 6].

Until recently, the effects imposed by numerous risk factors on the GD development have remained equal. Due to advances in immunosuppressive therapy, many immunological factors have lost their relevance [6]. However, given the modern trends in transplantation aimed at identifying and developing immunological tolerance and, thereby, justifying the possibility of minimizing immunosuppressive therapy (IST), immunological criteria associated with the development of donor organ dysfunction raise an increasing interest. For example, recent studies have indicated that early withdrawal from corticosteroids in recipients with GD may not be justified [7].

In recent years, we have witnessed the publication of nomograms and predictive indicators in the field of kidney transplantation (KT) using a variety of predictors to anticipate the donor organ dysfunction. In a number of studies, nomograms have been developed to predict the glomerular filtration rate one year after surgery, however, a significant number of predictors (18) may limit the widespread use of nomograms in

clinical practice [5]. The inclusion of a large set of predictors is also suggested by another study based on the use of the UNOS/OPTN (United Network for Organ Sharing/Organ Procurement and Transplantation Network) database, and using 20 GD variables to predict.

These studies can be compared with a simpler approach proposed by scientists who have developed nomograms for predicting donor organ dysfunction using a much smaller set of predictors: cold ischemia time, patient's age and weight, HLA-DR mismatch, preexisting antibody level and donor age [6, 8].

As a result, one of the main problems in transplantation is the discovery of simple, reliable and non-invasive markers that will make it possible to predict unfavorable immune responses and timely adjust IST in organ donor recipients. A reflection of the systemic nature of the ongoing processes is a change in the composition of some specific receptors on the immune system cells. When immunologically monitoring the recipients after allotransplantation, a wide range of immunological parameters is assessed; however, the identification of the most informative and significant ones for study in the post-transplant period remains an important trend in modern immunology.

The study objective was to establish immunological criteria for predicting renal graft dysfunction.

Material and methods

The study was conducted at the base of the State Institution "Republican Research Center for Radiation Medicine and Human Ecology" (RRS RM&HM), Gomel. A study group was formed of 197 kidney transplant recipients with end-stage chronic renal disease who underwent kidney allotransplantation in the Surgical Department (for

Transplantation, Reconstructive and Endocrine surgery) of the State Institution "Republican Research Center for Radiation Medicine and Human Ecology". The clinical study was conducted in conformity with the Helsinki Declaration of 1975 and approved by the Ethics Committee of the RRC RM&HE State Institution (Proceedings No. 5 dated 02.12.2013).

The inclusion criteria for 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.

The patients were divided into two groups according to the type of renal allograft function namely, 101 patients with primary graft function (PGF), and 96 patients with early GD. The early RG function was assessed by the level of blood creatinine and the need for dialysis on day 7 after surgery. The graft function was considered primary (PGF) at creatinine values below 300 μmol/L; and with creatinine values equal to or exceeding 300 μmol/L, or if dialysis was required in the first week after transplantation, the patients were allocated to the GD group [9]. Ninety healthy volunteers participated as a comparison group.

There were 122 men (61.9%) and 75 women (38.1%) among the RG recipients. The average age of the patients was 45.9 ± 0.9 years [95% confidence interval (CI) 44.1; 47.57]. Cold ischemia time was 12.38 ± 0.3 hours [95% CI 11.8; 13.0]. Before transplantation, 79.7% of patients were on program hemodialysis, and 18.78% were on peritoneal dialysis; 3 patients (1.5%) were at pre-dialysis stage. As for the period of receiving dialysis, the following patient distribution was noted: 33 patients (16.8%) for 5 years or longer, 116 (58.9%) patients for 1 to 5 years, and 45 (22.8%) for up to 1 year.

All patients received immunosuppressive therapy according to the

clinical kidney transplantation protocols (Appendix 1 to Order No. 6 issued by the Republic of Belarus Healthcare Ministry dated 05.01.2010).

Immunological investigation was performed on post-transplant day 3. To determine the expression of lymphocyte surface markers by flow cytometry, the sample preparation was performed according to the wash-free technology using monoclonal antibodies (Beckman Coulter and BD, USA) in the volumes recommended by the manufacturer.

Statistical data processing of the results was carried out using the Statistica 10.0 software package. Descriptive statistics of qualitative variables are presented as absolute and relative rates, and the quantitative statistics is presented in the format of the mean (M) [95% confidence interval] [CI -95%; + 95%] and median (Me) [interquartile range] [Q25; Q75]. Nonparametric criteria (Mann-Whitney U Test, Wilcoxon Matched Pairs Test) were used to determine the differences between groups in the levels of quantitative variables. For nominal variables, epy contingency table analysis was used to assess the frequency differences using the Pearson Chi-square (χ^2) test and Fisher's exact test (Pearson Chi-square, Fisher exact test). The correlation between the variables was determined using the Spearman Rank Order Correlations. To assess the parameters and their combinations when predicting the RG function, simple and multiple logistic regressions with stepwise parameter inclusion and exclusion (Forward and Backward Stepwise, respectively) were used. The quality of the logistic regression equation was assessed taking into account the statistical significance for the equation as a whole, the Nagelkerke R-squared value, the ability to reclassify observations, and the Hosmer-Lemeshow goodness of fit test. In addition, the significance of the regression equation coefficients and the normality of remainder distribution were assessed.

When creating a scoring system to predict the RG function, a

categorical regression procedure was used. The quality of the categorical regression equation was assessed taking into account the statistical significance for the equation as a whole ($p < 0.05$), the R-squared value, and the significance of the calculated coefficients of the regression equation. The Importance values for the model coefficients were used to determine the scores assigned to the parameters. The results were considered statistically significant at a significance level lower than 0.05.

Results and discussion

The patients of the compared groups had no statistically significant differences in gender, age, type of dialysis before transplantation, and ischemia time, as presented in Table 1.

Table 1. Comparative characteristics of patients

Group	Age, years M CI [-95%;+95%]	Gender, n (%)	Dialysis type n (%)	Ischemia time, hours M CI [-95%;+95%]
Total (n=197)	45.9 [44.1; 47.6]	Female 75(38.1%), Male 122(61.9%)	HD 157(79.7%) PD 37(18.8%) PtD 3(1.5%)	12.38 hours [11.8; 13.0]
PGF (n=101)	45.5±1.3 [42.9; 48.0]	Female 38 (37.6%) Male 63(62.4%)	HD 76(75.2%) PD 23(22.8%) PtD 1(2.00%)	11.9 hours [11.1; 12.8]
GD (n=96)	46.3±1.2 [43.9; 48.7]	Female 37 (38.5%) Male 59 (61.5%)	HD 81(84.4%) PD 14(14.6%) PtD 2(1.00%)	12.79 hours [11.9; 13.7]
Comparison between PGF and GD parameters	p=0.772 Mann–Whitney U-test	p=0.506 Fisher exact p, one-tailed	p=0.274 Pearson Chi-square	p=0.145 Mann–Whitney U-test

Before surgery, creatinine values did not differ significantly between the groups ($p = 0.032$), while in patients with GD, the urea level was statistically significantly lower compared to patients with PGF ($p = 0.0001$). The creatinine and urea on postoperative day 7 were statistically

significantly lower with PGF than with the GD development, which could be explained by the study design (Table 2).

Table 2. Biochemical parameters in patients (Me [Q25; Q75])

Parameter	Day	Primary Graft Function	Graft Dysfunction	p, Mann–Whitney U-Test
Creatinine, μmol/L	0	687.00 [579.00;932.00]	818.00 [627.00;997.00]	$_{PGF/GD}$=0.032
	7	148.50 [115.50;197.00]	525.00 [360.00;707.00]	$_{PGF/GD}$ <0.0001
Urea, mmol/L	0	19.00 [15.20;21.30]	15.80 [10.60;19.00]	$_{PGF/GD}$ =0.0001
	7	10.30 [7.80;14.50]	22.90 [17.30;34.40]	$_{PGF/GD}$ <0.0001

At the first stage of the study, the nonparametric Mann – Whitney test was used to assess the differences between the immunological parameters of the selected patient groups depending on the graft function on day 7. Also, on day 7, the Spearman correlations were assessed between all analyzed immunological parameters and the creatinine level.

Of all the immunological parameters, between which statistically significant differences were observed, 9 parameters with the minimum value of the Z-criterion were identified (Table 3).

Table 3. The results of a comparative analysis of the immunological parameters between the primary graft function and graft dysfunction groups

Immunological parameters	U Mann–Whitney U-Test	W Wilcoxon test	Z	p-value
$CD3^+CD8^+CD69^+$, %	53.500	3293.500	-10.261	<0.001
$CD3^+CD4^-CD8^-$, %	68.500	3308.500	-9.920	<0.001
$CD3^+CD127^+$, %	126.500	3207.500	-9.909	<0.001
$CD3^+CD4^+CD69^+$, %	136.500	3062.500	-9.796	<0.001
$CD3^+CD4^+$HLA-DR^+, %	253.500	3493.500	-9.492	<0.001
$CD19^+IgD^+CD27^-$, %	125.500	1895.500	-9.458	<0.001
$CD3^+CD4^+CD8^+$, %	352.500	2698.500	-9.111	<0.001
$Lym71^+$, 10^9/л	109.500	2125.500	-9.105	<0.001
$CD19^+CD40^+$, %	143.000	1574.000	-8.630	<0.001

Based on the results of the correlation analysis, there were 9 parameters also identified, for which a statistically significant correlation with the creatinine level on day 7 was determined with the maximum modulus value of the Spearman correlation coefficient, the results are presented in Table 4.

Table 4. Correlation analysis of immunological parameters on day 3 and creatinine blood level on day 7

Immunological parameters	Spearman's correlation coefficient	p-value
$CD3^+CD127^+$, %	+0.745608	<0.001
$CD3^+CD4^+CD69^+$, %	+0.742719	<0.001
$CD3^+CD4^-CD8^-$, %	+0.738619	<0.001
$CD3^+CD8^+CD69^+$, %	+0.710220	<0.001
$CD19^+IgD^+CD27^-$, %	-0.687203	<0.001
$CD3^+CD4^+CD8^+$, %	-0.686814	<0.001
$CD3^+CD4^+HLA\text{-}DR^+$, %	+0.658460	<0.001
$CD3^+CD4^+CD38^+$, %	-0.648654	<0.001
$CD19^+IgD^-CD27^+$, %	+0.573618	<0.001

Thus, summarizing the results from tables 3 and 4, we selected 11 immunological parameters for further stages of the study (Table 5).

Table 5. Immunological parameters in the studied groups

Immunological parameters	Comparison group	Primary graft function	Graft dysfunction
$CD3^+CD8^+CD69^+$, %	7.90 [5.45;12.00]	6.71 [5.33;8.74]*	15.22 [12.13;19.20]
$CD3^+CD4^-CD8^-$, %	3.50 [3.10;7.20]	1.86 [1.57;2.16]*	4.24 [3.10;4.72]
$Lym71^+$, 10^9/л	0.02 [0.01;0.03]	0.01 [0.01;0.03]	0.25 [0.17;0.53] *
$CD3^+CD4^+CD69^+$, %	9.70 [7.10;15.80]	4.45 [2.58;6.74]*	16.35 [13.78;21.44]
$CD3^+CD127^+$, %	82.60 [80.80;87.20]	82.90 [81.10;84.90]	89.59 [87.31;91.80]*
$CD19^+IgD^+CD27^-$, %	50.90 [44.00;62.90]	81.08 [77.06;84.38]*	52.20 [47.63;58.24]
$CD3^+CD4^+HLA\text{-}DR^+$, %	15.90 [7.40;22.60]	8.54 [5.58;10.12]*	24.43 [15.50;28.49]
$CD3^+CD4^+CD8^+$, %	1.40 [0.90; 3.80]	1.40 [0.94;1.82]	0.53 [0.42;0.63]
$CD19^+CD40^+$, %	97.10 [93.80;98.50]	86.20 [82.20;90.70]*	96.35 [94.53;98.90]
$CD19^+IgD^-CD27^+$, %	22.80 [19.10;27.70]	15.28 [12.11;18.44]*	31.68 [29.24;33.23]
$CD3^+CD4^+CD38^+$, %	45.20 [42.30;52.10]	52.78 [47.81;57.74]*	36.51 [30.47;45.90]

Note: * p <0.05 versus the parameters in the Comparison group

Considering the data obtained during the study, in Table. 5 we have shown the immunological parameters of peripheral blood, by which the studied patient groups were different, and which could serve as prognostic markers suggestive of a GD development.

The parameters identified in the previous steps were then individually assessed using logistic regression, calculating 2 Log-likelihood, Nagelkerke's R-squared value, percentage of correctly classified cases, and the area under ROC curve (AUC) with 95% confidence interval [95% CI]. For all logistic regression models, good prognosis characteristics were determined in relation to patient's status on day 7 (Table 6).

Table 6. Assessment of immunological parameters using logistic regression

Immunological parameters	- 2 Log-Plausibility	Nagelkerke R-Squared	Percentage of correctly classified cases	Odds ratio	AUC [95%CI]
$CD3^+CD8^+CD69^+$, %	32.178	0.92	95.9	10.347	0.990 [0.958-0.999]
$CD3^+CD4^-CD8^-$, %	38.129	0.90	92.3	1.02E+04	0.986 [0.950-0.998]
$Lym71^+$, 10^9/л	39.159	0.88	96.0	3.04E+42	0.972 [0.926-0.993]
$CD3^+CD4^+CD69^+$, %	47.768	0.87	95.8	2.323	0.974 [0.932-0.993]
$CD3^+CD127^+$, %	54.112	0.85	89.0	5.012	0.976 [0.936-0.994]
$CD19^+IgD^+CD27^-$, %	52.963	0.84	94.2	0.762	0.973 [0.930-0.993]
$CD3^+CD4^+HLA\text{-}DR^+$, %	69.086	0.80	89.2	2.072	0.953 [0.906-0.981]
$CD3^+CD4^+CD8^+$, %	85.089	0.74	92.6	0.001	0.935 [0.883-0.969]
$CD19^+CD40^+$, %	69.319	0.73	89.2	1.739	0.960 [0.907-0.987]
$CD19^+IgD^-CD27^+$, %	108.921	0.64	90.7	1.296	0.887 [0.825-0.933]
$CD3^+CD4^+CD38^+$, %	112.654	0.62	79.1	0.784	0.890 [0.828-0.935]

Note: the level of difference significance was $p < 0.001$ in all cases

As shown in Table 6, good predictive characteristics for graft function on day 7 were identified for all logistic regression models.

To predict the RG function, an attempt was made to create a model

based on the multiple logistic regression equation.

The selected indicators were analyzed for the presence of cross-correlation according to Spearman. To build a predictive model, we preferably used the combinations of parameters between which no strong correlation was observed (the value of Spearman's rank correlation coefficient was less than 0.7 in modulus).

Considering the above, at the first stage, the first 6 of 11 parameters presented in Table 6 were used as predictive variables to build up the model. As a result of applying the step-by-step method of parameter inclusion and exclusion (forward and backward stepwise), the equation that included two parameters $CD3^{+}CD4^{-}CD8^{-}$ and $CD19^{+}IgD^{+}CD27^{-}$ was build up, which was characterized by a high Nagelkerke R-squared value (0.990), by the ability to correctly reclassify observation (99%), and a significance level of $p > 0.05$ when checking the Hosmer–Lemeshow goodness of fit test. However, the fact that the calculated regression equation coefficients were statistically insignificant, as well as the abnormal distribution of the residuals, indicated the model instability.

To build a more stable model, we consistently applied the logistic regression with an alternate replacement of the parameters having the lowest scores while simultaneously using them to build an equation. The standard logistic regression model is as follows:

$P = 1 / (1 + Exp (-Y))$

where P is the probability of referring a patient to a risk group,

Y is the constant $B+a_1X_1+a_2X_2+a_nX_n$

a_n stands for regression coefficients (B for each value)

X_n stands for the values of the parameters included in the model.

As a result, two equations with good prognostic characteristics and statistically significant coefficients were obtained (using two pairs of

parameters $CD19^{+}IgD^{+}CD27^{-}$ and $CD3^{+}CD4^{+}CD8^{+}$ and $D19^{+}IgD^{+}CD27^{-}$ and $CD3^{+}$ $CD8^{+}CD69^{+}$), as presented in Table 7, 8.

Table 7. Parameters of logistic regression equation for $CD19^{+}IgD^{+}CD27^{-}$ and $CD3^{+}CD4^{+}CD8^{+}$ variables

Immunological parameters	B	Root Mean Square Error	Wald	Value	Exp (B)	95% CI for EXP(B)	
						Lower	Upper
$CD19^{+}IgD^{+}CD27^{-}$	-0.326	0.098	11.186	0.001	0.722	0.596	0.951
$CD3^{+}CD4^{+}CD8^{+}$	-4.636	1.458	10.111	0.001	0.010	0.001	54.310
Constant	26.759	7.688	12.115	0.001	4.183E+11		

Notes: B, the regression equation coefficient; Wald, Wald test for the significance of B coefficient for the corresponding independent variable, Value, statistical significance by using Wald test

Meanwhile, the equation based on the pair $CD19^{+}IgD^{+}CD27^{-}$ and $CD3^{+}CD4^{+}CD8^{+}$ differed in the level of significance $p < 0.05$ when checking the Hosmer–Lemeshow goodness of fit test; all that did not confirm its good quality.

Table 8. Parameters of logistic regression equation for $CD19^{+}IgD^{+}CD27^{-}$ and $CD3^{+}CD8^{+}CD69^{+}$ variables.

Immunological parameters	B	Root Mean Square Error	Wald	Value	Exp (B)	95% CI for EXP(B)	
						Lower	Upper
$CD19^{+}IgD^{+}CD27-$	-0.204	0.079	6.730	0.009	0.815	0.699	0.951
$CD3^{+}CD8^{+}CD69^{+}$	1.990	1.023	3.789	0.052	7.319	0.986	54.310
Constant	-5.385	10.182	0.280	0.597	0.005		

Notes: B, the regression equation coefficient; Wald, Wald test for the significance of B coefficient for the corresponding independent variable, Value, statistical significance by using Wald test

Thus, when using the results shown in Table. 8, a logistic regression equation was compiled, which can be used to estimate the likelihood of developing GD, taking into account the level of $CD19^+IgD^+CD27^-$ and $CD3^+CD8^+CD69^+$ lymphocyte subpopulations:

$$P = 1 / (1 + EXP (- (- 5.4 + (-0.2 \times CD19^+IgD^+CD27^-) + (2 \times CD3^+CD8^+CD69^+))))$$

At $P > 0.5$, a GD is predicted; and at $P \leq 0.5$, a PGF is predicted.

When using the ROC analysis procedure, the diagnostic characteristics of the model were estimated based on the logistic regression equation (Fig. 1). We found that the resulting model had the sensitivity of 97.2%, specificity of 97.7%, accuracy 97.4%, positive predictive value (PPV) of 0.986, and negative predictive value (NPV) of 0.955.

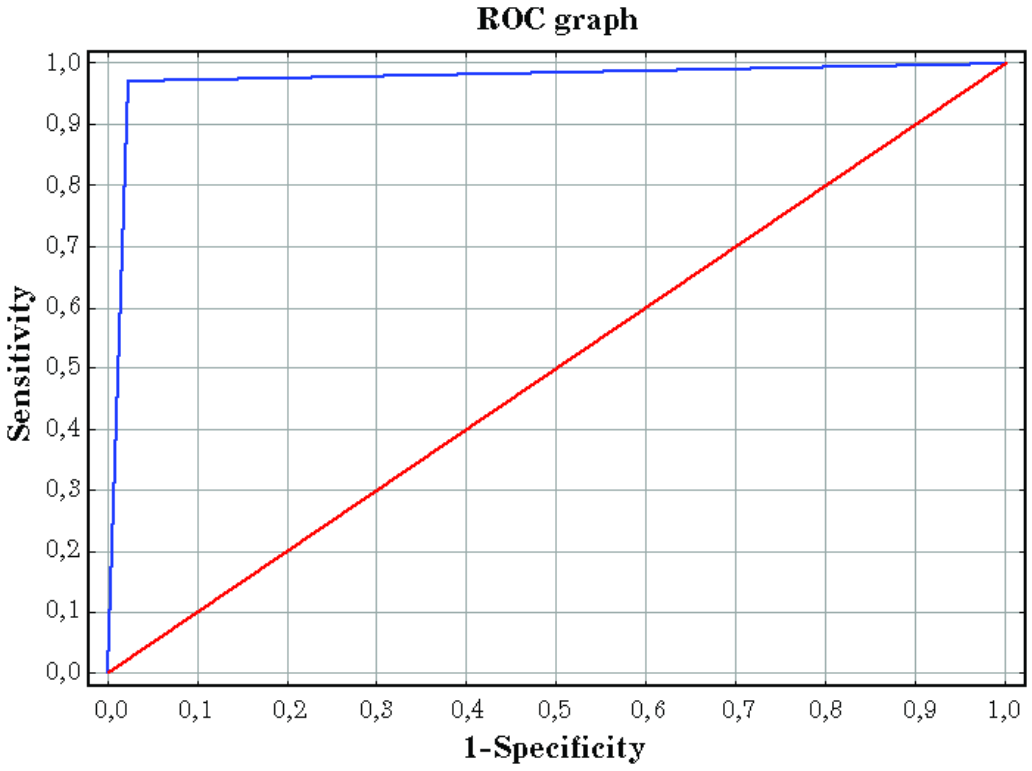

Fig. 1. Diagnostic characteristics of the model for predicting a renal graft dysfunction

The multiple logistic regression equation based on the pair of parameters $CD19^+IgD^+CD27^-$ and $CD3^+CD8^+CD69^+$ was characterized by a Nagelkerke's R-squared value of 0.943, the ability to reclassify well the observations (97.4%), and a significance level of p = 0.962 when testing the Hosmer-Lemeshow goodness of fit test.

However, an abnormal remainder distribution was observed for the resulting model, which could indicate the model instability. Therefore, an attempt was made to create a scoring system for predicting the renal graft status on day 7 based on the pair of parameters $CD19^+IgD^+CD27^-$ and $CD3^+CD8^+CD69^+$.

When using the ROC analysis procedure, the $CD19^+IgD^+CD27^-$ ≤72.7% and $CD3^+CD8^+CD69^+$>9.7% values were selected as cut-off points. Additional binary variables were created by reencoding $CD19^+IgD^+CD27^-$ ≤72.7% to 0 and $CD3^+CD8^+CD69^+$ > 9.7% to 1.

The categorical regression method was used to determine the values for the scores to be assigned. As a result, the model quality parameters were calculated: the multiple one R 0.939 and R-squared 0.881; the level of significance for the model as a whole and for the model coefficients was less than 0.05. The Importance indices for the re-encoded variables $CD19^+IgD^+CD27^-$ and $CD3^+CD8^+CD69^+$ were 0.54 and 0.46, respectively, which indicated approximately equal significance of the variables for prediction.

Thus, a scoring system was created to predict the RG function; according to this system, the values of the parameters $CD19^+IgD^+CD27^-$ (IgD^+naive B lymphocytes) and $CD3^+CD8^+CD69^+$ activated T-lymphocytes were determined.

With a $CD19^+IgD^+CD27^-$ value not exceeding 72.7%, 1 point is assigned, and with a level of more than 72.7%, 0 point is assigned. If the value of $CD3^+CD8^+CD69^+$ exceeds 9.7%, 1 point is assigned, and if the

level of $CD3^+$ $CD8^+CD69^+$ is not exceeding 9.7%, 0 point is assigned. The total score (TS) is determined by summing up the scored points.

TS = Score 1 + Score 2.

With a value of TS equal to zero, the PGT is predicted, and with a TS equal or lower 1, the GD is predicted.

We have evaluated the predictive characteristics of the scoring system using the ROC analysis procedure. Thus, the system had the sensitivity of 91.9%, the specificity of 100%, the accuracy 94.9%, PPV was 1, and NPV was 0.877 (Fig. 2).

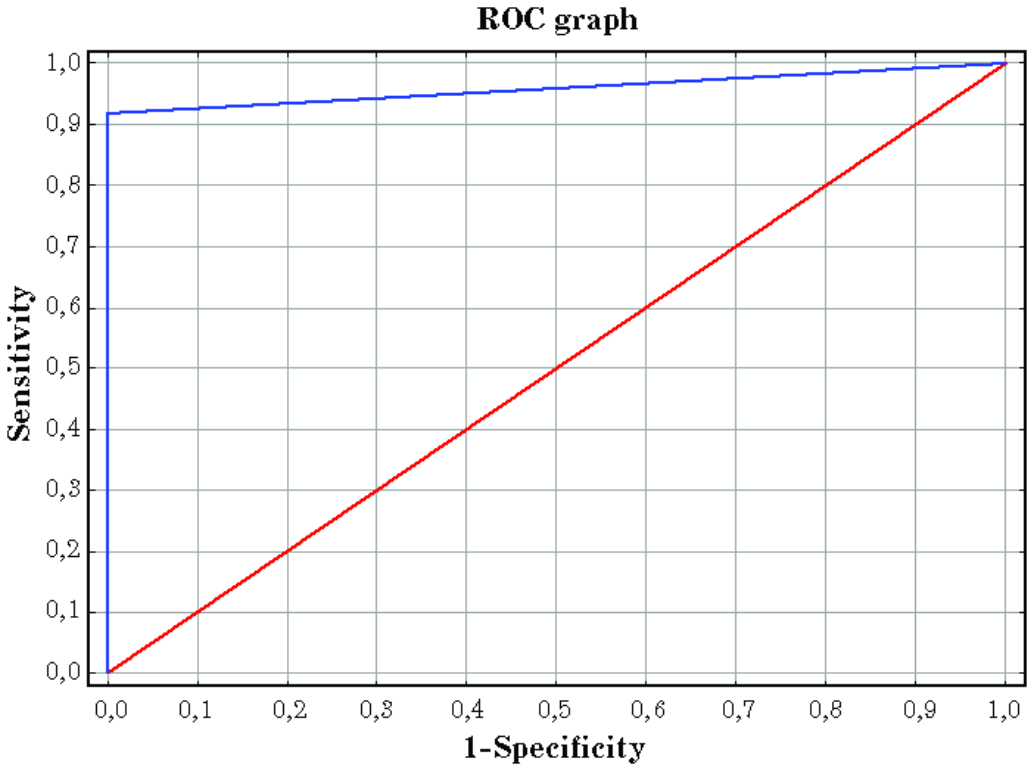

Fig. 2. Diagnostic characteristics of the scoring system in predicting a renal graft dysfunction

The results obtained were confirmed in previous studies: for example, when studying the mechanisms of tolerance in renal transplantation, we revealed that in patients with a higher tolerogenic

potential among subpopulations of B-lymphocytes, an increase in the number of transitional and naive B lymphocytes was revealed with a decrease in memory B lymphocytes [10-12]. In turn, it was shown that the level of $CD8^+$lymphocytes expressing the CD69 receptor correlated with the development of acute RG rejection [13].

Thus, the above results can serve as a scientific justification for the feasibility of the proposed method for predicting GD. In clinical practice, it can be used for the timely prevention of donor organ dysfunction.

Conclusions

1. Immunological markers $CD19^+IgD^+CD27^-$ and $CD3^+CD8^+CD69^+$ can be used to predict a renal graft dysfunction.

2. The proposed method allows prediction of a renal allograft dysfunction with a sensitivity of 91.9%, a specificity of 100% and an accuracy of 94.9% at values of $CD19^+IgD^+CD27^-$ not exceeding 72.7% and $CD3^+$ $CD8^+CD69^+$ over 9.7% on the 3rd postoperative day.

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