

# Neutrophil-lymphocyte ratio and creatinine reduction ratio predict good early graft function among adult cadaveric donor renal transplant recipients. Single institution series

## Authors' Contribution:

A – Study Design  
B – Data Collection  
C – Statistical Analysis  
D – Data Interpretation  
E – Manuscript Preparation  
F – Literature Search  
G – Funds Collection

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## ABSTRACT:

The Background Delayed graft function (DGF) is a common complication following kidney transplantation and is associated with ischemia-reperfusion injury (IRI). Lymphocytes contribute to the pathogenesis of IRI and ischemia-reperfusion related to delayed graft function.

**Materials and Methods:** 135 Caucasian patients received a kidney graft from deceased heart-beating organ donors. We divided patients into 2 groups - patients with the eGFR $\geq$ 30 on the 21st day post-transplantation (n=36) and patients with the eGFR $<$ 30 on the 21st day post-transplantation (n=99) to assess kidney graft function. We measured serum creatinine levels on 1st and 2nd post-transplant day and preoperative levels of monocytes, lymphocytes, platelets, and neutrophils and their ratios.

**Results:** We have found statistically significant differences between the eGFR $<$ 30 and the eGFR $\geq$ 30 groups in the average lnLymphocytes (0.36 +/-0.6 vs. -0.016 +/-0.74, respectively p=0.004) lnNLR (1.27 +/-0.92 vs. 1.73+/-1.08 p=0.016) lnLMR (1.01 +/-0.57 vs. 0.73 +/-0.64 p=0.02), lnPLR (4.97 +/-0.55 vs. 5.26 +/- 0.67 p=0.023), and CCR 2% (-20.20 +/- 21.55 vs. -4.29 +/- 29.62 p=0.004). In the univariate analysis, factors of lnLymphocytes  $\geq$ 0.22 (OR=0.331 95%CI 0.151-0.728 p=0.006), lnLMR $\geq$ 1.4 (OR=0.255 95%CI 0.072-0.903 p=0.034) were associated with a worse graft function, while lnNLR $\geq$ 1.05 (OR=2.653 95%CI 1.158-6.078 p=0.021), lnPLR $\geq$ 5.15 (OR=2.536 95%CI 1.155-5.566 p=0.02) and CRR2 (OR=3.286 95% CI 1.359-7.944 p=0.008) indicated a better graft function.

**Conclusion:** A higher absolute lymphocyte count (lnLymphocytes) and lnLMR, as well as lower lnNLR and lnPLR were associated with a lower eGFR on the 21st day after kidney transplantation. In the multivariate analysis, CRR2 in combination with either lnLymphocytes, lnNLR or lnPLR improved the accuracy of detecting patients with poor graft function.

## BACKGROUND

The neutrophil-lymphocyte ratio (NLR) is an easily applicable method for evaluating systemic inflammation. Inflammatory response plays a crucial role in ischemia - reperfusion injuries (IRI) of kidney grafts. Therefore, it may influence the early results of transplantation, especially delayed graft function (1-2).

Delayed graft function is a common complication following cadaveric kidney transplantation. In some studies with various DGF definitions, the incidence of delayed function is as low as 20% and as high as 70.8% (3-4). Delayed graft function results from IRI, along with many contributing factors, e.g., cold ischemic time, warm ischemic time, donor type, organ storage, as well as the age of the donor (5,6). One of the most important factors determining DGF is ischemic-reperfusion injury, which leads to the production of free radicals and other toxic metabolites causing inflammatory injury of the tissue. The severity of the injury may be associated with not only donor-related factors, but especially systemic inflammatory response of the recipient (7,8). Changes in relative levels of circulating leukocytes provide a means to measure this response.

The creatinine reduction ratio on the second after kidney transplantation measures kidney impairment and predicts the renal function in the future (7-9). Our present study is the first one to

use the ratios deriving from preoperative blood morphology results, such as NLR and CRR2 to detect improvement in patients with better early graft function.

In this study, we evaluated retrospectively preoperative levels of monocytes, lymphocytes, platelets, and neutrophils, and their ratios in a group of 135 renal transplant recipients (from cadaveric donors, after brain death diagnosis, stored in simple cold ischemia, without mechanical perfusion). Serum creatinine levels were measured on the 0, 1st, 2nd and 21st day after ktx and the eGFR (estimated glomerular filtration rate) had been calculated according to the MDRD. We calculated serum creatinine reduction ratios on the 2nd day after ktx. Patients who needed dialysis before the 2nd-day measurement of creatinine were excluded from the study. A retrospective analysis of the clinical data was performed.

## MATERIALS AND METHODS

We conducted a single centre retrospective study at the Department of General and Transplant Surgery, Medical University of Lodz. Between September 2011 and April 2015, 135 Caucasian patients received a kidney graft from deceased heart-beating organ donors. We divided patients into 2 groups - patients with the eGFR $\geq$ 30 on the 21st day post-transplantation (n=36) and

**Tab. I.** Recipients characteristics. All differences between groups were non-significant ( $p>0.05$ ).

	EGFR 21ST DAY < 30	EGFR 21ST DAY > 30
n	99	36
Age, yr	45.41 (+/-13.7)	43.83 (+/-13.07)
male	56 (56.6%)	15 (41.7%)
female	43 (43.4%)	21 (58.3%)
BMI	25.12 (+/-3.96)	24 (+/-3.1)
<b>Immunosuppression</b>		
Tac, MMF, Steroids	69 (69.7%)	28 (77.8%)
Cyc, MMF, Steroids	20 (20.2%)	4 (11.1%)
Basiliximab, Tac, MMF, Steroids	10 (10.1%)	4 (11.1%)
WIT [min]	21.5 (+/-5.17)	21 (+/-6.5)
CIT [min]	1285.72 (+/-417.6)	1123.27 (+/-420.72)

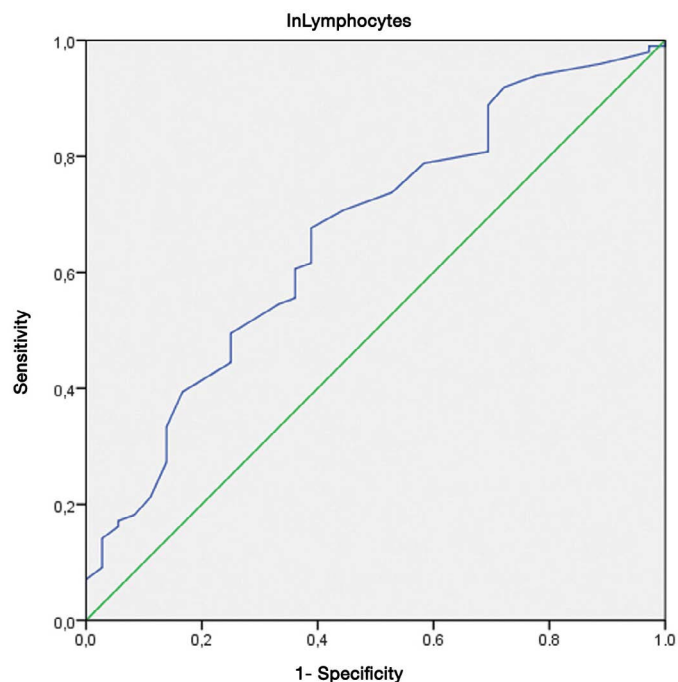
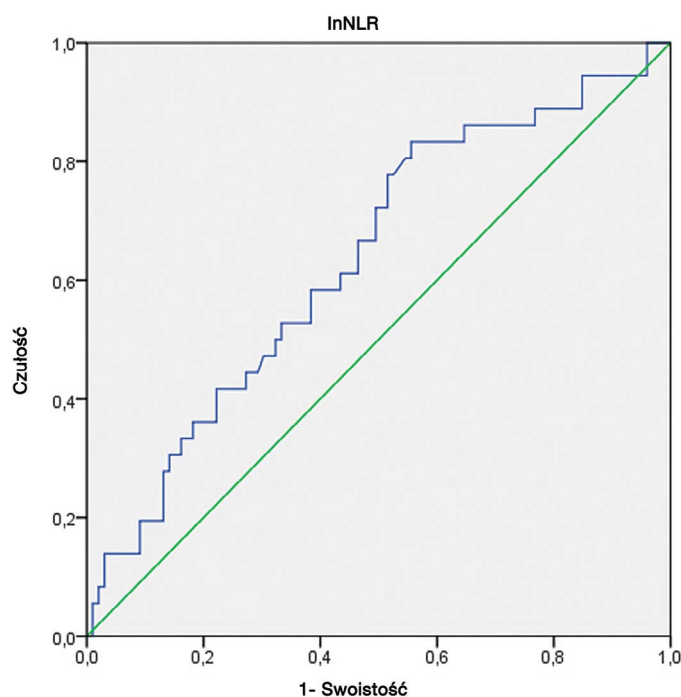
patients with the  $eGFR<30$  on the 21st day post-transplantation ( $n=99$ ) to assess kidney graft function. Moreover, we measured the serum creatinine levels on the 1st and 2nd post-transplant days. As for predictors, we took into account pre-transplantation BMI, age, gender, cold ischemic time (CIT), warm ischemic time (WIT) and the type of immunosuppression. Blood morphology results were obtained shortly before KTx and the administration of the first dose of the immunosuppression regimen. All patients participating in the study underwent hemodialysis or peritoneal dialysis before Ktx and before the measurement of initial creatinine and WBC levels.

Using these results, we calculated the neutrophil to lymphocyte ratio (NLR), neutrophil to monocyte ratio (NMR), platelet to lymphocyte ratio (PLR), lymphocyte to monocyte ratio (LMR) and creatinine reduction ratio, which was calculated according to  $CRR2(\%) = ((Cr1st\ day - Cr2nd) \times 100\%) / Cr1st\ day$ . The glomerular filtration rate was calculated according to the Modification of Diet in Renal Disease (MDRD)  $eGFR = 186 \times creatinine^{-1.154} \times age^{-0.203} \times 1.212$  (if Afro-American)  $\times 0.742$  (if female) (12). These parameters were then transformed using a natural logarithm. Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp. Armonk, NY). A p value less than 0.05 was considered statistically significant.

## RESULTS

The basic descriptive statistics of the groups are shown in Table 1. There were no significant differences between groups concerning mean age, BMI, WIT or CIT. Similarly, both groups were comparable in gender and immunosuppression regimen type distributions. All patients in the study groups received a standardized immunosuppression regimen consisting of calcineurin inhibitors: tacrolimus or cyclosporine, mycophenolate mofetil or mycophenolate sodium and steroids. In a few cases, induction with Basiliximab was administered at 20 mg before transplantation and 20 mg on the 4th day after.

When analysing blood morphology parameters, there were statistically significant differences between the  $eGFR<30$  and the

**Fig. 1.** ROC curve – prediction of good graft function on the 21st day after ktx using preoperative InLymphocytes.**Fig. 2.** ROC curve – prediction of good graft function on the 21st day after ktx using preoperative InNLR.

$eGFR \geq 30$  groups in the average InLymphocytes ( $0.36 \pm 0.6$  vs.  $-0.016 \pm 0.74$  respectively  $p=0.004$ ) InNLR ( $1.27 \pm 0.92$  vs.  $1.73 \pm 1.08$   $p=0.016$ ) InLMR ( $1.01 \pm 0.57$  vs.  $0.73 \pm 0.64$   $p=0.02$ ), InPLR ( $4.97 \pm 0.55$  vs.  $5.26 \pm 0.67$   $p=0.023$ ) and CCR 2% ( $-20.20 \pm 21.55$  vs.  $-4.29 \pm 29.62$   $p=0.004$ ). No statistically significant differences were observed for neutrophils, platelet, monocytes counts ( $5.94$  vs.  $6.59$   $p=0.524$ ;  $213,56$  vs.  $197,83$   $p=0.107$ ;  $0.57$  vs.  $0.53$   $p=0.376$ ) (Table 2). In the univariate analysis, factors of InLymphocytes  $\geq 0.22$  (OR=0.331 95% CI 0.151-0.728  $p=0.006$ ), InLMR  $\geq 1.4$  (OR=0.255 95% CI 0.072-0.903  $p=0.034$ ) were associated with a worse graft function, while InNLR  $\geq 1.05$  (OR=2.653

Tab. II. Logistic regression models summary

					MULTIVARIATE ANALYSIS WITH CRR2		
					Adjusted OR	95% CI	p
	Cut-off point	OR	95%CI	p			
lnLymphocytes	$\geq 0.22$	0.331	0.151-0.728	0.006	0.308	0.153-0.62	0.001
lnLMR	$\geq 1.4$	0.255	0.072-0.903	0.034	N/A		
lnNLR	$\geq 1.05$	2.653	1.158-6.078	0.021	1.985	1.223-3.221	0.006
lnPLR	$\geq 5.15$	2.536	1.155-5.566	0.02	1.158	1.037-6.078	$<0.0001$
lnPLR	N/A	3.286	1.359-7.944	0.008	N/A		

95% CI 1.158-6.078  $p=0.021$ ), lnPLR $\geq 5.15$  (OR=2.536 95% CI 1.155-5.566  $p=0.02$ ) and CRR2 (OR=3.286 95% CI 1.359-7.944  $p=0.008$ ) indicated a better graft function.

Moreover, ROC curves built for lnLymphocytes and lnNLR, these being the strongest predictors of satisfactory and inadequate graft function respectively, had AUC=0.636 (95%CI 0.531-0.741  $p=0.016$ ) and AUC=0.664 (95%CI 0.561-0.767  $p=0.004$ ) for distinguishing patients with the eGFR $\geq 30$  from the ones with the eGFR $<30$ .

ROC curves for models of combined lnLymphocytes + CRR2, lnNLR + CRR2 and lnLMR+CRR2 improved accuracy in the detection of a worse graft function, e.g.: lnLymphocytes + CRR2 had AUC=0.724 (95%CI 0.627 -0.822,  $p=0.001$ ), lnNLR + CRR2 had AUC=0.726 (95%CI 0.625-0.827,  $p=0.004$ ) and lnLMR+CRR2 had AUC=0.709 (95%CI 0.610-0.808,  $p=0.004$ ).

## DISCUSSION

In the present study, we report an inexpensive and simple method of identifying patients at risk of delayed graft function in the early period after kidney transplantation. In the univariate analysis, a higher total lymphocyte count of lnLymphocytes, as well as lower lnNLR were associated with a lower eGFR on the 21st day after kidney transplantation. A combination of lnLymphocytes, lnNLR, lnLMR and CRR2 reduction correlates with the 21st-day serum creatinine and the eGFR in transplant recipients, and it allows for early detection of recipients with worse graft function in the early period after ktx.

Lymphocytes contribute to the pathogenesis of ischemia-reperfusion injury and ischemia-reperfusion-related delayed graft function. In kidney models of IRI lymphocytes mediated inflammatory response as well as the tissue repair processes. Renal ischemia-reperfusion injury is associated with the initiation of an inflammatory response. In turn, the secretion of pro-inflammatory cytokines and chemotactic substances attracts monocytes and neutrophils, which then differentiate into macrophages and dendritic cells, activating the adaptive immune response (13,14,15). The main source of both monocytes and lymphocytes is the spleen. In the Wystrychowski et al. (16) case study, the entire kidney function preservation was superior in rats that underwent splenectomy together with renal ischemia, and the survival rate was two times higher in that group in comparison with controls. A lower amount of lymphocytes and monocytes is probably associated with a decreased immune

Tab. III. Sensitivity and specificity of preoperative Lymphocytes, lnNLR, lnPLR in combination with CrR2 in detection of patients with good graft function on the 21st day after ktx.

	CUT OFF POINT	SENSITIVITY	SPECIFICITY
Lymphocytes+CrR2	0.242	80.6%	51.4%
lnNLR+CrR2	0.209	75%	54.7%
lnPLR+CrR	0.208	77.8%	52.6%

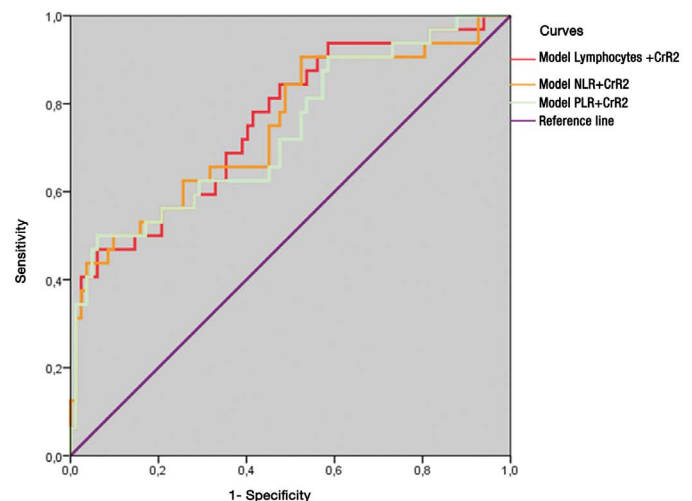


Fig. 3. ROC curve – prediction of good graft function on the 21st day after ktx using preoperative Lymphocytes, lnNLR, lnPLR in combination with CrR2.

response leading to smaller ischemia-reperfusion injury. Savransky et al. (17) investigated a model of IRI in T cell receptor (TCR) depleted mice. The  $\alpha\beta$ -TCR-deficient mice were protected from serologic kidney injury 24 hours after ischemia-reperfusion, as measured by serum creatinine, compared to WT mice.

That growing evidence suggests that lymphocytes serve a crucial role in IRI in renal transplant subjects.

In the Turkmen et al. (18) case study, NLR was elevated in the group of renal transplant recipients when compared to healthy individuals, and it may be related to ongoing inflammatory processes in the group of patients with end-stage renal disease undergoing renal transplantation.

Interestingly, our results differ from that obtained by Halazun et al. (19) having examined the relationship between NLR and DGF.

In the Halazun et al. case study, elevated preoperative recipients NLR levels were associated with higher chances of developing DGF after kidney transplantation. However, that study considered recipients of unknown ethnicity, including children. In particular, different types of kidney grafts were used, e.g. from donors after brain death, donors after circulatory death, as well as living donors. The authors also do not mention the type of cold storage of kidneys before the transplant. All these factors may have hampered the results.

Moreover, this study examined only the occurrence of DGC defined as a requirement for dialysis within the first week after transplantation, unless this was performed for hyperkalemia. Otherwise, the authors didn't assess the function of graft in the early postoperative period or in the long-term perspective.

Similarly, Çankaya E et al. (20) reported that patients with end-stage renal disease have higher NLR when compared with healthy controls in general. Specifically, individuals who received preemptive kidney transplantation had significantly lower NLR one year after the transplantation than non-preemptive patients.

It should also be noted that in the both aforementioned studies, the authors didn't report whether the differences in the absolute lymphocyte count or neutrophils count underlie the observed difference in NLR, or whether this phenomenon was accompanied by other statistically significant differences in blood morphology.

In this study, we report that the absolute lymphocyte count (ALC) and creatinine reduction ratio between the 1st and 2nd postoperative days are the most important factors predicting graft function in the short-term postoperative period. Indeed, differences in NLR, PLR, LMR between study groups are directly connected with the difference in the ALC. As these "derivatives" didn't prove to be superior in predicting graft function, we hypothesize that monitoring the lymphocyte count preoperatively might emerge as a useful factor in assessing graft function. Moreover, in this clinical setting, its combination with CRR2 yielded relatively high sensitivity, making the assessment of these factors even more promising.

To our knowledge, there are a few studies reporting the significance of monitoring ALC in terms of kidney transplantation.

As per our knowledge, up to date there are no studies assessing ALC impact on graft function, except for studies conducted by Weissenbacher A et al. (21). In this study, the authors report a retrospective analysis of 430 kidney transplantations. The patients received the induction with either alemtuzumab or basiliximab. The analysis showed that the ALC count correlated with CMV status and DGF development in the alemtuzumab group. Patients with CMV-IgG(+) status had a higher ALC prior to kidney transplantation, while patients who developed DGF had a higher lymphocyte count within the first 3 weeks after the procedure, than the patients without normal graft function.

It might be hypothesized that ALC prior to KTx partially reflects changes in the phenotype and the activity of lymphocytes subpopulations, that have an impact on the development of acute renal injury or delayed graft function. The other possible hypothesis that might be considered is that patients with a lower preoperative

ALC are more likely to achieve proper immunosuppression in the postoperative period, thus their immunological response doesn't trigger mechanisms leading to kidney injury. In terms of the research into the pretransplant immunological marker of ischemic-reperfusion related to acute kidney injury, there is a great number of studies showing a correlation between lymphocytes subpopulations frequencies, both circulating and intragraft, or their function and the risk of development of impaired graft function or acute graft rejection (22,23,24).

Nguyen et al. (25) performed a prospective study, in which recipients of donor deceased kidney graft had their peripheral blood CD4+CD25hiFoxP3+ regulatory lymphocyte T (Treg) frequency measured by flow cytometry. Moreover, Treg suppressive function was also assessed. Afterwards, the patients were divided into DGF, SGF (slow graft function) and IGF (immediate graft function) groups. The authors reported no statistically significant difference between Treg frequencies between groups. However, they proved that pretransplantation Treg suppressive function was significantly lower in DGF and SGF groups in comparison with the IGF group. They also noted that Treg function was a significant predictor of eGFR at the 14th post-transplantation day. Recently, the same research group (26) showed that specifically the preoperative percentage of CD4+CD127lo/-TNFR2+ Treg cells was significantly lower in SGF and DGF groups. This phenomenon was also accompanied by the decrease of absolute Treg quantity.

In view of these facts, it would be interesting to validate our data on a bigger group of patients and simultaneously analyze certain lymphocyte subpopulations in terms of their quantity and activity.

Interestingly, in the Reddan et. al. case study, a higher lymphocyte count was associated with higher serum albumin and creatinine levels, as well as a lower age and black race in a group of 44,114 patients receiving hemodialysis due to end stage renal disease. A high neutrophil count was associated with lower serum albumin and creatinine, younger age and white race (all Ps <0.0001). (27)

Our study is based on a homogenous group, which consist of middle-aged adult Caucasian cadaveric donors and recipients only.

The 30 mL/min/1.73 m<sup>2</sup> eGFR cut-off was chosen because it is a border value in the classification of chronic kidney disease. According to KDOQI, patients with GFR lower than 30 mL/min/1.73 m<sup>2</sup> are in the 3rd stage, and GFR higher than 30 mL/min/1.73 m<sup>2</sup> reclassify to the 4th stage.

Since the epidemiology of post-transplantation chronic kidney disease can be altered by methods of calculating GFR, we chose the MDRD method of eGFR calculation, as in the early period after kidney transplantation, it is associated with relatively low understimulation rates and in general, it is the most commonly used (28). Moreover, the MDRD calculation is superior for low values of GFR calculation (30) and is also recommended for GFR estimations by the National Kidney Foundation – KDOQI guidelines (30, 29).

On the other hand, one must consider the fact, that there are many DGF definitions, what is more proper graft function in the early post-transplantation isn't strictly defined. DGF is the most widely described as a need for hemodialysis in the first week after ktx. However, according to the Decruyenaere et al. case study, all dialy-



sis-based definitions of DGF are associated with a high sensitivity and low specificity for identifying patients with DGF and serum creatinine based ones are more heterogeneous (31). In our study, we have proposed our own definition of early good graft function by measuring the serum creatinine level on the 21st day after ktx and calculating eGFR. Our end point from a clinical point of view was chosen by the fact that it may be correlated with the length of hospital stay and not directly with a defined DGF itself. However, in our study, the group with a eGFR lower than 30 is relatively large, and it may be associated with a relatively high CIT and low rates of induction-based immunosuppression used in the study group. However, in some studies, eGFR lower than 10 mL/min/1.73 m<sup>2</sup> is a threshold for DGF diagnosis. With this cut-off point, the DGF group is relatively smaller in our study (32). In some studies with various DGF definitions, the incidence of delayed function is as low as 20% and as high as 70.8% (3-5).

Our cut-off value of eGFR > 30. detects a large group of patients with poor graft function but is not equal to DGF itself according to the vast definition.

It should be acknowledged that a small number of patients constitutes the limitations of our study. Therefore, the results should be confirmed by a large multicenter prospective study.

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