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>=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>=30 groups in the average lnLymphocytes (0.36 +/-0.6 vs. -0.016 +/-0.74, respectively p=0.004) InNLR (1.27 +/-0.92 vs. 1.73+/1.08 p=0.016) InLMR (1.01 +/-0.57 vs. 0.73 +/-0.64 p=0.02), InPLR (4.97 +/-0.55 vs. 5.26 +/-0.67 p=0.023), and CCR 2% (-20.20 +/-29.62 p=0.004). In the univariate analysis, factors of lnLymphocytes >=0.22 (OR=0.331 95%CI 0.151-0.728 p=0.006), InLMR=1.4 (OR=0.255 95%CI 0.072-0.903 p=0.034) were associated with a worse graft function, while lnNLR>=1.05 (OR=2.536 95%CI 1.58-6,078 p=0.021), InPLR>=5.15 (OR=5.849 95%CI 1.152-5.66 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 InLMR, as well as lower lnNLR and InPLR were associated with a lower eGFR on the 21st day after kidney transplantation. In the multivariate analysis, CRR2 in combination with either lnLymphocytes, InNLR or InPLR improved the accuracy of detecting patients with poor graft function.

KEYWORDS: Neutrophil-lymphocyte ratio, creatinine reduction ratio, graft function, cadaveric donor, delayed graft function kidney transplant

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 also 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>=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. 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)x100%)/Cr1st day. The glomerular filtration rate was calculated according to the Modification of Diet in Renal Disease (MDRD) eGFR=186 x creatinine-1.154  x age-0.203 x 1.212 (if Afro-American) x 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, mycofenolate mofetil or mycofenolate 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 eGFR>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. 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 lnLymphocytes >=0.22 (OR=0.331 95% CI 0.151-0.728 p=0.006), lnLMR>=1.4 (OR=0.255 95% CI 0.072-0.903 p=0.034) were associated with a worse graft function, while lnNLR>=1.05 (OR=2.653
95% CI 1.158-6.078 p=0.021), lnPLR>=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>=30 from the ones with the eGFR<30. ROC curves for models of combined lnLymphocytes + CRR2, lnNLR + CCR2 and lnLMR+CCR2 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 + CCR2 had AUC=0.726 (95%CI 0.625-0.827, p=0.004) and lnLMR+CCR2 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, lnNLRI, lnLMR and CCR2 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 response leading to smaller ischemia-reperfusion injury. Savran- sky et al. (17) investigated a model of IRI in T cell receptor (TCR) depleted mice. The αβ-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 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 hypothesized 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 for 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 infection in terms of kidney transplantation, while patients who developed DGF had a higher 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 m2 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 m2 are in the 3rd stage, and GFR higher than 30 mL/min/1.73 m2 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 underestimation 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-
six-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 m2 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.

REFERENCES:
22. Hueso M, Mestre M, Benavente Y, Bas J, Grinyó JM, Navarro E. Pretransplant low CD3+CD25 high cell counts or a low CD3+CD25 high/CD3+HLA-DR+ ratio are associated with an increased risk to acute renal allograft rejection. Transplantation. 2011; 92(5):536-42

In our study, we do not take into consideration acute rejections are associated with a high sensitivity and low specificity for identifying patients with DGF and serum creatinine based ones are more heterogeneous. 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 m2 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).

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CONCLUSION

In this study, we report a simple and inexpensive method of identifying patients at risk of poor graft function in the early period after kidney transplantation. In the univariate analysis, a higher absolute lymphocyte count (InLYmphocytes) and lnLMR, as well as lower lnNLR and lnPLR were associated with lower eGFR on the 21st day after kidney transplantation. In the multivariate analysis, CRR2 in combination with either InLYmphocytes, lnLMR or lnPLR may improve the accuracy of detecting patients with early poor graft function. Since the combination of InLYmphocytes and CRR2 yields the greatest AUC, we hypothesize that further research into lymphocytes subpopulations that take into account ALC and CRR2 may reveal the significance of these factors in the development of ischemia-reperfusion related AKI.


