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# Potential Utility of Neutrophil-to-Lymphocyte, Platelet-to-Lymphocyte, and Neutrophil, Lymphocyte, and Platelet Ratios in Differential Diagnosis of Kidney Transplant Acute Rejection: A Retrospective, Propensity Score Matched Analysis

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Data Collection B  
Statistical Analysis C  
Data Interpretation D  
Manuscript Preparation E  
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**Background:** Acute kidney transplant rejection can negatively affect long-term graft function. The neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio have been proposed as non-invasive predictors of acute rejection in stable kidney transplant recipients. The aim of this study was to validate the predictive value of these ratios, as well as neutrophil, lymphocyte, and platelet ratios in the diagnosis of acute rejection during the early post-transplant period.

**Material/Methods:** After propensity score matching, we compared 71 kidney recipients with biopsy-proven acute rejection with 71 patients without rejection and also subjects with different histologic types of rejection. All 3 types of blood cell count-derived ratios were calculated 6 and 3 days prior to biopsy and on the day of biopsy.

**Results:** There were 15 patients with T cell-mediated rejection, 33 with vascular rejection, and 23 with antibody-mediated rejection. The values of all examined ratios did not differ between subgroups with and without rejection. However, at all post-transplant study time-points, patients with antibody-mediated rejection had significantly higher values of all analyzed ratios than subjects with other types of rejection. In multivariate regression models, higher values of blood cell count-derived ratios were independently associated with the occurrence of antibody-mediated rejection.


**Conclusions:** In the early post-transplant period, the values of neutrophil-to-lymphocyte, platelet-to-lymphocyte, and neutrophil, lymphocyte, and platelet ratios were similar in patients with and without an acute rejection episode, but significantly higher values were found in subjects with antibody-mediated rejection as compared with other types of rejection and those without rejection. High values of analyzed ratios in patients with satisfactory early kidney graft function may be helpful in selecting subjects with increased risk of subclinical antibody-mediated rejection.

**Keywords:** Biopsy, Fine-Needle • Graft Rejection • Kidney Transplantation

**Abbreviations:** **AR** – acute rejection; **ABMR** – antibody-mediated rejection; **Bx** – kidney graft biopsy; **CIT** – cold ischemia time; **CRP** – C-reactive protein; **DGF** – delayed graft function; **ECD** – extended criteria donor; **IGF** – immediate graft function; **KTRs** – kidney transplant recipients; **N/LP** – neutrophil, lymphocyte and platelet ratio; **NLR** – neutrophil-to-lymphocyte ratio; **PLR** – platelet-to-lymphocyte ratio; **PRA** – panel-reactive antibodies; **ROC** – receiver operating characteristics; **SGF** – slow graft function; **TCMR** – T cell-mediated rejection; **VR** – vascular rejection

**Full-text PDF:** <https://www.annalsoftransplantation.com/abstract/index/idArt/937239>



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## Background

Kidney transplantation is the optimal method of treatment in patients with end-stage renal disease, however, acute rejection (AR) is one of main complications, which worsens the long-term graft function and patient survival [1,2]. Early kidney graft dysfunction can also be caused by delayed graft function, infection, nephrotoxicity, or surgical complications; however, kidney graft biopsy is usually performed in such patients during differential diagnosis. In contrast, in subjects with satisfactory early graft function, the ongoing subclinical rejection may be only diagnosed based on the early protocol biopsy [3]. The protocol biopsy program is not universally adopted, and in the transplant centers with such an approach, the timing of kidney graft protocol biopsies can vary widely, from the first post-transplant hospitalization to 12 months after transplantation [4].

Needle biopsy of the transplanted organ with subsequent histological evaluation remains the criterion standard for AR diagnosis. However, this procedure can be seriously complicated by hematoma, urinary bladder obstruction, need for blood transfusions or surgical procedure, graft loss, or even death [5,6], although major complications are rare if the biopsy is performed by an experienced operator under the guidance of an imaging method [7]. Moreover, various medical contraindications often present in the early post-transplant period may make this procedure challenging. Additional biopsy shortcomings like sampling errors and inter-observer variability furtherly limit the accuracy of this method. Therefore, different alternative non-invasive procedures were recently developed and tested, comprising imaging techniques (contrast media-enhanced ultrasound and magnetic resonance imaging), novel urine and serum biomarkers and microarray molecular analysis [8-11]. Additionally, a deep-learning computer-aided diagnostic system, based on the fusion of both imaging markers and clinical biomarkers, was reported to have high accuracy in early detection of AR in kidney transplant recipients (KTRs) [12].

In recent years, the utility of various blood cell count-derived ratios, such as neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR), in the diagnosis of AR has been examined, based on the at least partly inflammatory nature of the acute rejection process [13,14]. Both reports described the significant differences in NLR values between stable KTRs groups of patients with and without AR, but the latter analysis unexpectedly founded NLR and PLR values that were several times higher in the rejection-free patients. Interestingly, neutrophil, lymphocyte, and platelet ratio (N/LP) were found to be associated with acute kidney injury after major abdominal surgery [15]. Thus, we performed a retrospective study to analyze the utility of inflammatory markers calculated from the individual types of cells counted in peripheral blood in the

differential diagnosis of AR during the early period after kidney transplantation.

## Material and Methods

### Study Group

We retrospectively analyzed all consecutive KTRs in our center from January 2013 to October 2020, in whom an AR episode was diagnosed based on the kidney graft biopsy performed during the first post-transplant hospital stay, which is the period from transplantation procedure to discharge from the hospital. Patients were identified in the center-operated prospective transplant register. The study was conducted in accordance with the Declaration of Helsinki. According to the opinion of the Bioethics Committee of the Medical University of Silesia (PCN/CBN/0022/KB/164/21), issued July 12, 2021, the present analysis based on anonymous patient data was permitted without obtaining individual informed consent.

Out of 898 KTRs, acute rejection during the first post-transplant hospital stay was diagnosed in 72 patients (8.0%). The control group consisted of patients without an early AR episode, transplanted during the same period of time. As there were substantial differences across the whole analyzed period concerning the percentage of highly-immunized patients or the structure of induction therapy algorithms, the control group without acute rejection was selected based on individual propensity scores, described in detail in the statistical section below.

### Immunosuppression Regimen

Basically, triple immunosuppressive therapy, consisting of tacrolimus, mycophenolate mofetil, or sodium, and steroids, was used in all patients. The initial target tacrolimus levels were set at 5-15 ng/ml and did not change during the study period. At hospital discharge and thereafter, the target tacrolimus level was 6-12 ng/ml. The initial dose of mycophenolate was 500 mg BID or 750 mg BID, depending on patient's baseline weight. During the operating procedure, 500 mg of methylprednisolone was given i.v., then 125 mg at the first post-operative day and then 20 mg of oral prednisone if the patient was on basiliximab induction. In subjects undergoing antithymocyte globulin induction, the dosing of oral prednisone after the ATG cessation was 20 mg/day, then was decreased to reach 10-15 mg/day at discharge.

### Kidney Graft Function

Immediate graft function (IGF) was defined as the serum creatinine concentration ( $S_{Cr}$ ) at day 3 post-transplant below or

equal to 3 mg/dl, slow graft function (SGF) as  $S_{Cr}$  above 3 mg/dl at day 3, and delayed graft function (DGF) requiring dialysis therapy during the first week after transplantation. Of note, we also classified subjects as developing DGF in cases of polyuria, high serum creatinine levels, and non-continuous kidney graft Doppler spectrum during the first post-operative days, despite the lack of need for dialysis therapy.

### Kidney Graft Biopsies, Acute Rejection Classification, and Treatment

Kidney graft biopsies were usually performed between the 8<sup>th</sup> and 10<sup>th</sup> post-transplant days. Between January 2013 and September 2015, biopsy was performed in patients with DGF that did not subside during the first week ( $n=11$ ). Since October 2015, the protocol biopsies were introduced in our center in all patients, except those with clinical contraindications and those who did not give consent. During this period, 60 AR episodes were diagnosed. Throughout the whole study period, prior to the biopsy procedure, all subjects gave informed consent. Samples were stained with standard hematoxylin and eosin, periodic acid Schiff (PAS), methenamine silver, and trichrome. All biopsies were assessed according to the contemporary Banff recommendations. Patients with borderline changes were treated similarly to grade 1A T cell-mediated rejection (TCMR). All biopsy specimens were evaluated by the same experienced histopathologist. Based on the results of kidney histology and additional information (eg, presence of donor-specific antibodies, C4d staining) AR episodes were classified as tubulointerstitial (TCMR grade IA/B), vascular (VR, identical with TCMR grade IIA/B, III) or humoral, antibody-mediated rejection (ABMR). If there were only histologic features representative for T cell rejection without vascular lesions, patients received 3 methylprednisolone pulses at 500 mg/day. If intimal or transmural arteritis dominated in the histologic spectrum, but there was no clear evidence for antibody-mediated rejection (glomerulitis, PTC-itis), VR was diagnosed and patients were treated using ATG as the first-line treatment. Lastly, when ABMR was diagnosed, patients were initially treated with ATG, plasmaphereses and IVIG (if the presence of DSA was confirmed). Of note, since 2015 we introduced a novel protocol for an early AMBR treatment, based on plasmaphereses and bortezomib, with very promising mid-term results [16].

### Laboratory Data

The absolute white blood cell count, neutrophil count, lymphocyte count, and platelet count were extracted from complete blood counts taken: immediately before transplantation, 6 days before the biopsy (Bx-6), 3 days prior to biopsy (Bx-3), and at the day of biopsy (Bx). The NLR was calculated by dividing the absolute neutrophil count by the absolute lymphocyte count; the PLR was calculated by dividing the absolute platelet count

by calculated lymphocyte count (obtained by multiplying the absolute white blood cell count by the percentage of lymphocytes), and the N/LP ratio was calculated as:

$$(\text{neutrophil count} \times 100) / (\text{lymphocyte count} \times \text{platelet count}).$$

### Statistical Analysis

Statistical analyses were performed using STATISTICA 13.3 (Tibco, Inc., Palo Alto, CA, USA) and MedCalc version 20 (MedCalc Software, Ostend, Belgium). Data are presented as means with 95% confidence interval (95% CI), medians with Q25-Q75 quartile values, or frequencies. The subjects constituting the control group without acute rejection episode were selected out of 826 patients transplanted during the same time-span, based on 1: 1 matching with Caliper 0.2, using the “greedy” algorithm, based on individual propensity scores. The propensity scores were calculated, taking into account the following covariates: the recipient age, post-transplant follow-up period (defining the transplant era), number of transplantations, and the type of induction treatment used (none/basiliximab/antithymocyte globulin). All propensity score calculations were performed using SAS software (version 9.4, SAS Institute, Inc., Cary, North Carolina, USA). Finally, we used 2 matched cohorts of 71 patients with and 71 patients without acute rejection episode during the first post-transplant stay for all study analyses.

The initial comparison between these study subgroups was performed using the *t* test (for quantitative variables) or  $\chi^2$  test (for qualitative variables). Variables with skewed distribution were compared using the Mann-Whitney U test. Another analysis was performed between subgroups defined based on the type of acute rejection (TCMR, VR, ABMR) using the Kruskal-Wallis test or  $\chi^2$  test. In this analysis, between-subgroup statistics was performed using the Mann-Whitney U test with Bonferroni correction.

Receiver operating characteristics (ROC) analyses were applied to determine the cut-off values for NLR, PLR and N/LP values, associated with the occurrence of AMBR. Univariate analyses were performed for the occurrence of ABMR, including age, HLA mismatch, cold ischemia time (CIT), extended criteria donor (ECD) status, and retransplantation, as well as NLR, PLR, and N/LP values calculated at Bx and Bx-3 time-points. Multiple regression analyses were performed for the occurrence of ABMR as the dependent variable, including CIT, retransplantation, and each out of 6 evaluated blood count-based ratios as potential independent variables. The stepwise selection method was used. The significance level to allow a variable to enter into the model and to stay in the model was set at 0.2 and 0.3, respectively. In all statistical tests, *P* values below 0.05 were considered as statistically significant.

**Table 1.** Clinical characteristics of study subgroups with and without early acute rejection episode in the early post-transplant period.

Variable	Acute rejection n=71	No rejection n=71	Statistics
<b>Patient data</b>			
Recipients age [years]	51.4 (48.5-54.4)	51.7 (49.1-54.3)	0.89
Sex [M/F]	37/34	39/32	0.74
BMI [kg/m <sup>2</sup> ]	26.0 (25.0-26.9)	25.4 (24.5-26.4)	0.43
Dialysis vintage [months]*	31 (22-63)	29 (20-40)	0.27
Diabetes [n (%)]	9 (12.7)	13 (18.3)	0.36
Post-transplant observation time [months]	46 (41-51)	46 (41-51)	0.89
Last PRA >20% [n (%)]	6 (8.6)	6 (8.6)	1.0
<b>Transplantation data</b>			
Transplant No [1/2/3]	53/15/3	56/14/1	0.57
Donor age [years]	47.6 (44.4-50.8)	46.9 (43.9-49.9)	0.77
Donor serum creatinine >1.5 mg/dl [n (%)]	24 (33.8)	21 (29.6)	0.59
ECD status [n (%)]	22 (31.9)	20 (28.2)	0.71
CIT [h]	18.1 (16.5-19.7)	17.6 (15.9-19.3)	0.69
HLA class I mismatch	2.3 (2.1-2.6)	2.1 (1.9-2.3)	0.22
HLA class II mismatch	0.73 (0.59-0.88)	0.63 (0.49-0.77)	0.33
Induction therapy (ATG/IL-2RB/none)	19/38/14	17/39/15	0.92
Initial graft function (IGF/SGF/DGF)	11/33/27	15/34/22	0.57
Initial tacrolimus level [ng/ml]	14.1 (12.3-16.0)	13.2 (11.7-14.8)	0.47
Tacrolimus level at 7-9 POD [ng/ml]	7.9 (7.3-8.4)	8.4 (7.6-9.1)	0.27
Mycophenolate mofetil initial dose [g/day]*	1.0 (1.0-1.5)	1.0 (1.0-1.5)	0.98

Data presented as means with 95% confidence interval or \* medians with Q25-Q75 quartile values or frequencies. BMI – body mass index; PRA – panel-reactive antibodies; HLA – human leukocyte antigen; MICA – human major histocompatibility complex class I-related gene A; ECD – extended criteria donor; CIT – cold ischemia time; ATG – antithymocyte globulin; IL-2RB – interleukin-2 receptor blocker; IGF – immediate graft function; SGF – slow graft function; DGF – delayed graft function; POD – post-operative day.

## Results

### Study Subgroups

After the propensity score matching, 2 cohorts were compared: patients with biopsy-proven AR during the first post-transplant hospital stay (median duration of 24 (14-35) days) and patients without AR (median length of stay 15 (13-19) days;  $P<0.001$ ). The clinical characteristics of study subgroups are given in **Table 1**. Patients in both subgroups did not differ in sex, BMI, dialysis vintage, the occurrence of pre-transplant diabetes, cold ischemia time, or HLA mismatch. Potential kidney transplant candidates had been screened for the presence of panel-reactive antibodies (PRA) and anti-HLA antibodies (class I, class II, and anti-MICA). Out of all those parameters of pre-transplant immunologic status, the only difference was a significantly higher percentage of

anti-HLA class I antibodies in the AR subgroup (71.8 vs 42.3% in the control group;  $P<0.01$ ). Additionally, there were no significant differences regarding donor data and early kidney graft function, as well as initial immunosuppression measures within the first 2 post-transplant weeks. In the whole study cohort, the causes of chronic kidney disease were as follows: glomerulonephritis (39.4%), diabetic nephropathy (9.9%), pyelonephritis (8.5%), autosomal dominant polycystic kidney disease (11.3%), hypertensive nephropathy (10.6%), and other and unknown (20.3%). There were no significant differences in the structure of the pathogenesis of chronic kidney disease between the 2 study subgroups ( $P=0.55$ ).

### Kidney Biopsy Results

The first kidney graft protocol biopsy, which was the basis of AR diagnosis, was performed at a median of 9 (8-9) days

**Table 2.** Median values of NLR, PLR, and N/LP ratios calculated at all study time-points in subjects with and without acute rejection episode.

	Acute rejection (N=71)	No rejection (N=71)	p
<b>Neutrophil-to-lymphocyte ratio</b>			
Preoperatively	3.1 (2.3-4.7)	2.7 (1.9-4.7)	0.29
Bx-6	12.0 (6.3-29.3)	12.4 (6.0-26.0)	0.81
Bx-3	4.4 (2.9-12.0)	4.7 (2.8-13.1)	0.88
Bx	5.3 (2.9-12.1)	4.2 (2.5-10.2)	0.44
<b>Platelet-to-lymphocyte ratio</b>			
Preoperatively	133 (109-200)	158 (112-217)	0.31
Bx-6	251 (168-443)	263 (160-668)	0.78
Bx-3	168 (118-274)	169 (120-382)	0.47
Bx	182 (115-311)	170 (127-389)	0.76
<b>Neutrophil, lymphocyte and platelet ratio</b>			
Preoperatively	1.5 (0.9-2.2)	1.2 (0.8-2.1)	0.11
Bx-6	7.4 (3.5-19.6)	6.6 (3.0-14.6)	0.53
Bx-3	2.7 (1.2-8.6)	2.2 (1.3-10.2)	0.67
Bx	2.4 (1.0-6.3)	1.6 (0.9-4.8)	0.22

Bx-6 – 6 days prior to biopsy; Bx-3 – 3 days prior to biopsy; Bx – at the day of biopsy.

after transplantation. In the whole AR subgroup, there were 15 TCMR, 33 vascular, and 23 ABMR cases. In a rejection-free group, most patients (66.6% of the whole cohort) were biopsied at the same post-transplant time (between the 8<sup>th</sup> and 10<sup>th</sup> days), whereas in those transplanted before 2015, biopsy was not routinely performed except in subjects with DGF longer than 1 week.

The distribution of different types of AR significantly differed depending on the type of induction therapy (ATG vs IL2-RB vs none:  $\chi^2=32.9$ ;  $P<0.001$ ). There were significantly more TCMR and VR episodes in patients who were transplanted using IL2-RB (27.5% and 52.5%, respectively) or no-induction (21.4% and 71.4%, respectively), versus patients who received ATG induction (5.3% and 10.5%, respectively; all  $P<0.001$ ). However, ABMR episodes occurred significantly more frequently in patients with ATG induction (84.2%) than in the 2 other groups (15% in the IL2-RB group and 7.1% in the group without induction).

#### Blood Count-Based Ratios in Patients with and without AR Episode

**Table 2** presents median values of NLR, PLR, and N/LP calculated at all study time-points (preoperatively, Bx-6, Bx-3, and Bx). There were no significant differences between subjects

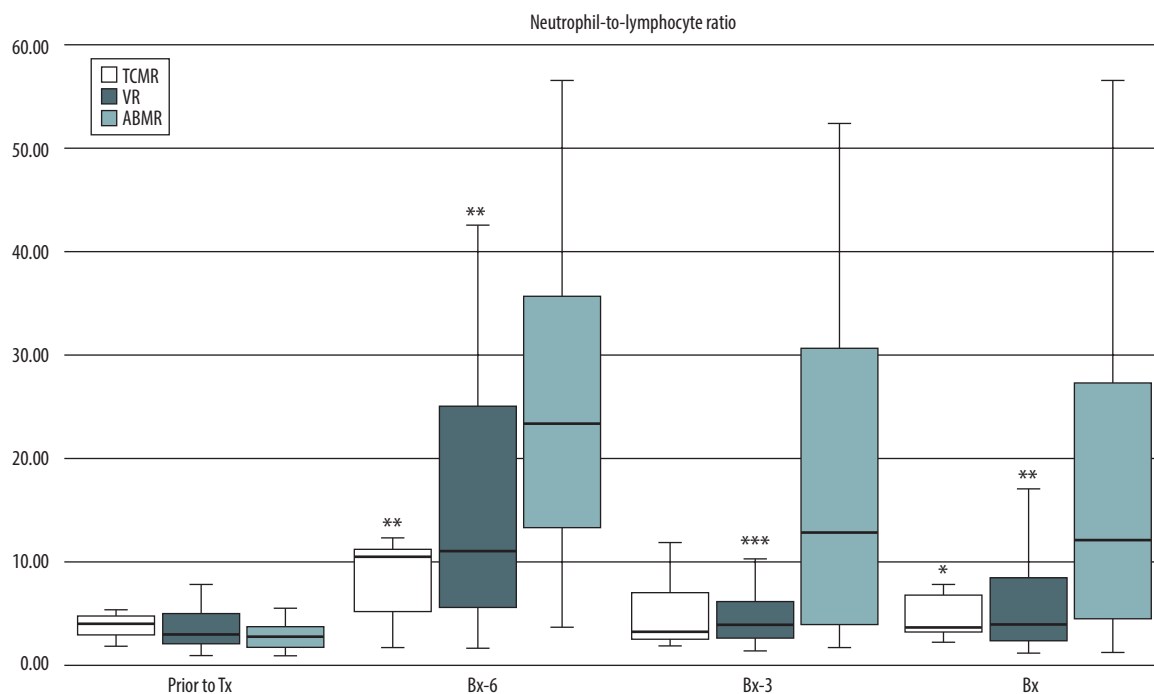
with and without AR. Of note, the concomitant C-reactive protein (CRP) values were also similar (data not shown). There was also no association between NLR, PLR, and N/LP values and concomitant CRP values in both study subgroups.

Prior to transplantation, median values of all 3 analyzed markers in the subgroups of patients with different type of induction therapy (ATG vs IL2-RB vs none) were similar. After transplantation, at all 3 study time-points, the highest values of NLR were noted in the ATG subgroup [Bx-6: 41.4 (27.4-59.0) vs 10.0 (5.5-13.8) in IL2-RB and 7.2 (4.5-20.2) in subgroup without induction; all  $P<0.001$ ; Bx-3: 30.6 (17.1-44.3) vs 3.7 (2.7-5.3) and 3.1 (2.3-5.1), respectively; all  $P<0.001$ ; Bx: 23.7 (13.9-46.0) vs 3.5 (2.4-4.8) vs 3.5 (2.1-6.4), respectively, all  $P<0.001$ ]. The analogous values of PLR and N/LP were also significantly higher in the ATG subgroup (data not shown).

#### Blood Count-Based Ratios in Patients with Different Types of Rejection

When considering the pre-transplantation values of all 3 ratios, they were comparable in the 3 analyzed subgroups (TCMR, VR, ABMR), except for significantly greater NLR in the TCMR subgroup vs the ABMR subgroup (4.1 (3.1-4.9) vs 2.7 (1.8-3.8);  $P<0.05$ ). However, at all post-transplant study time-points,





**Figure 1.** Pre-transplant and early post-transplant median values of NLR in kidney recipients with different types of acute rejection. *P* values as compared with the corresponding ABMR group; \* *P*<0.05, \*\* *P*<0.01, \*\*\* *P*<0.001. Figure created using STATISTICA 13.3 (Tibco Inc., Palo Alto, CA, USA).

including the day of kidney graft biopsy, patients with ABMR had significantly higher NLR than subjects with TCMR or VR [Bx-6: 24.4 (14.5-41.5) vs 10.6 (4.3-11.5) vs 11.1 (5.7-25.1), respectively; both *P*<0.01; Bx-3: 17.1 (3.7-32.0) vs 3.4 (2.3-7.7); *P*<0.01 vs 4.0 (2.8-5.7); *P*<0.001, respectively; Bx: 14.6 (3.8-28.4) vs 3.8 (3.2-7.2); *P*<0.05 vs 3.8 (2.4-8.4); *p*<0.01, respectively] (Figure 1).

Similarly, there were significantly higher PLR values in the ABMR subgroup than in the TCMR and VR subgroups [Bx-6: 440 (281-967) vs 198 (153-242); *P*<0.01 vs 236 (150-341); *P*<0.05, respectively; Bx-3: 356 (131-1158) vs 152 (121-179) vs 149 (108-196), respectively, both *P*<0.05; Bx: 311 (164-562) vs 164 (115-203) vs 170 (106-247), respectively, both *P*<0.05] (Figure 2).

Also, N/LP values were significantly higher in the ABMR subgroup than in the TCMR or VR subgroups [Bx-6: 17.8 (6.9-29.6) vs 5.1 (2.2-8.2) vs 5.8 (2.5-18.2), respectively; both *P*<0.01; Bx-3: 11.1 (2.7-23.6) vs 2.0 (1.2-4.1); *P*<0.01 vs 1.9 (1.2-3.6); *P*<0.001, respectively; Bx: 9.0 (2.8-21.8) vs 2.0 (1.1-2.9); *P*<0.05 vs 1.8 (0.9-3.6); *P*<0.001, respectively] (Figure 3).

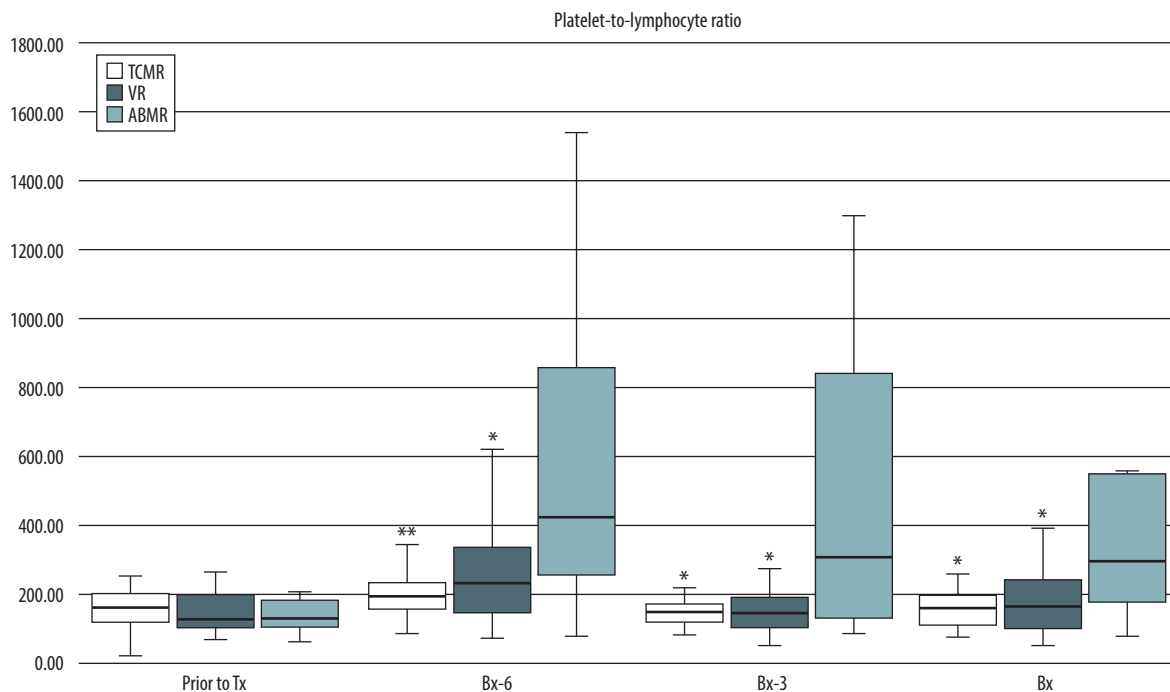
Notably, NLR, PLR, and N/LP values did not differ between the TCMR and VR subgroups, but they were significantly greater in the ABMR subgroup as compared with patients without AR

at Bx-3 and Bx time-points (data not shown), except for the PLR difference at the Bx-3 time-point (*P*=0.08).

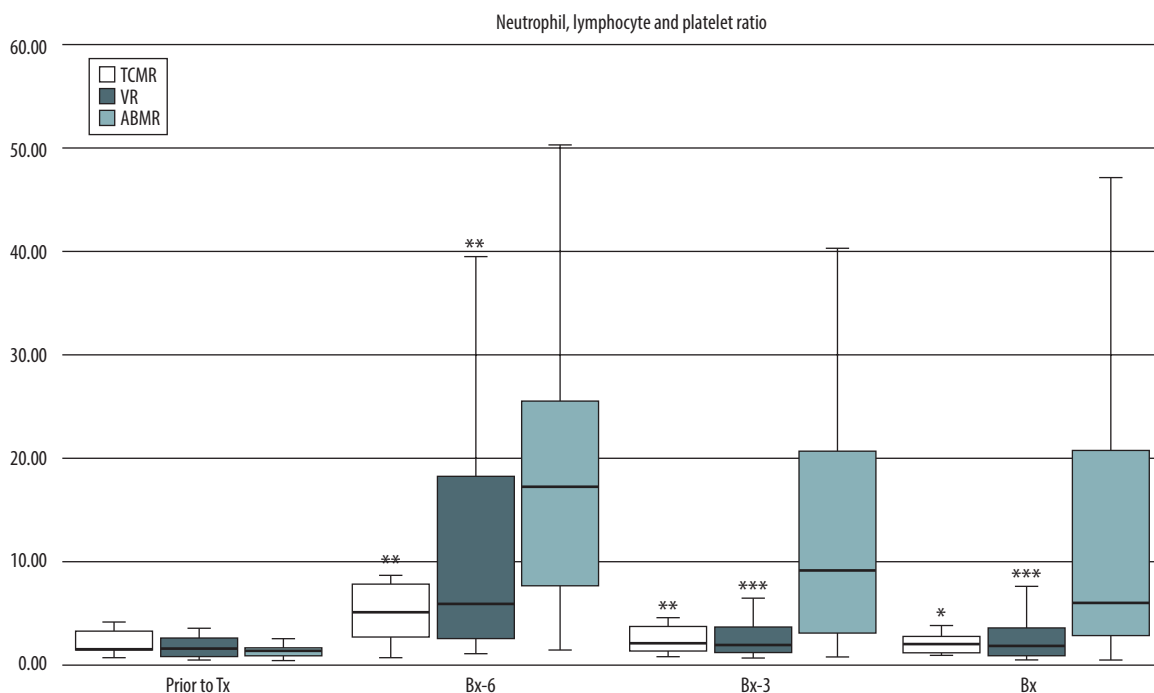
In the period from pre-transplant measurement to the 3<sup>rd</sup> post-transplant day (Bx-6 time-point), the median values of NLR increased by a significantly greater multiplier in ABMR [\*9.9 (6.1-20.4)] than in VR [\*3.5 (1.8-5.9); *P*<0.001] or TCMR [\*2.2 (1.2-3.7); *P*<0.02] subjects, respectively. Similarly, there was a significantly greater increase in PLR and N/LP values in ABMR [\*2.7 (1.0-6.5) and \*13.4 (7.5-30.2), respectively] than in VR [\*0.9 (0.7-1.3); *P*<0.02 and \*3.2 (2.0-7.0); *P*<0.001, respectively] or TCMR [\*0.9 (0.8-1.4); *P*<0.05 and \*3.4 (1.2-4.6); *P*<0.02, respectively] between those 2 time-points. There were no significant differences between the VR and TCMR groups.

### ROC Analyses

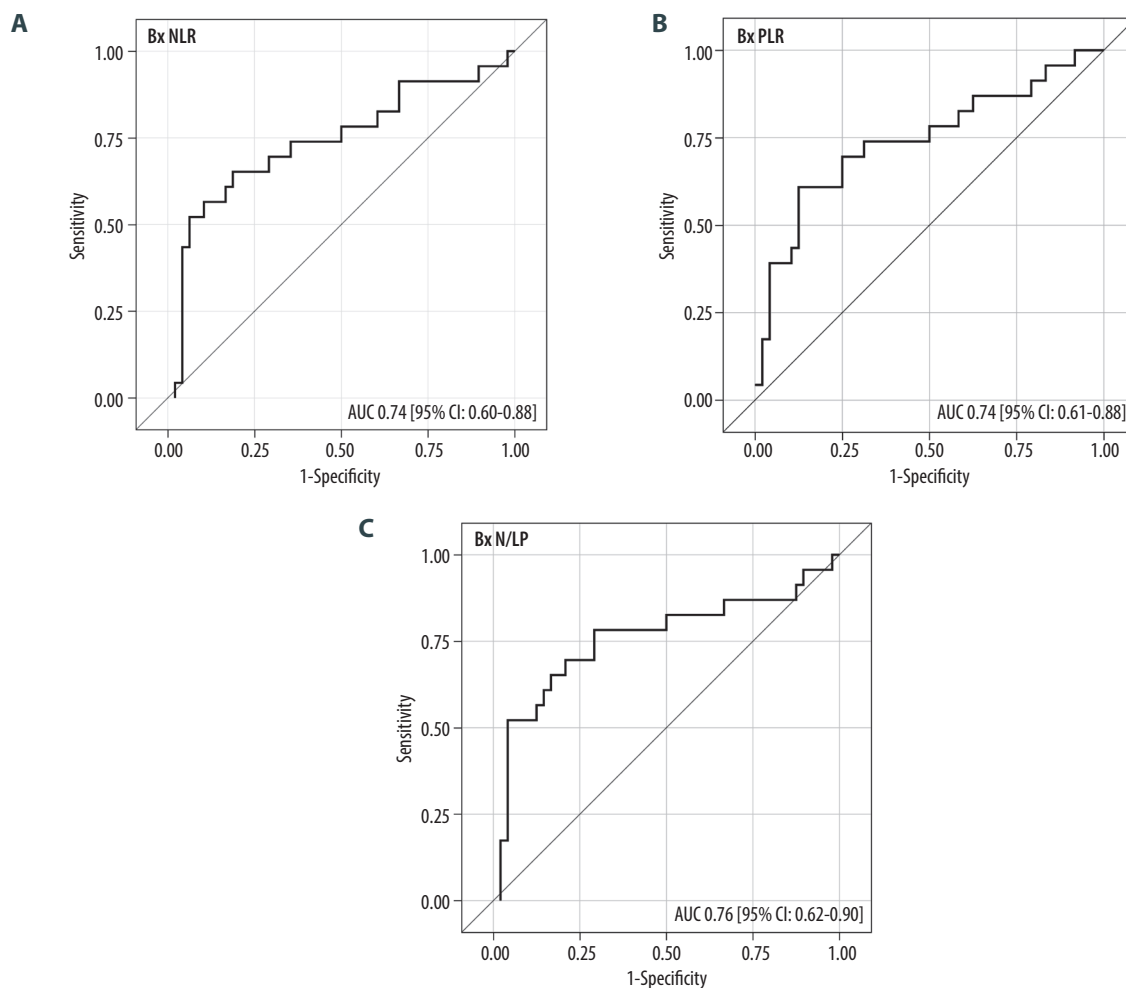
The ROC analysis for the Bx time-point revealed that NLR, PLR, and N/LP values above 8.7, 284, and 2.78, respectively, increased the risk for ABMR, with 65% (95% CI: 43-84) sensitivity and 81% (67-91%) specificity for NLR, 61% (39-80%) sensitivity and 88% (75-95%) specificity for PLR, and 78% (56-93%) sensitivity and 71% (56-83%) specificity for N/LP (Figure 4A-4C). Similar analyses for the Bx-3 time-point showed the following cut-off values: 8.26, 212, and 6.37 for NLR, PLR, and N/LP, respectively, with 70% (95% CI: 47-87%) sensitivity and 85%



**Figure 2.** Pre-transplant and early post-transplant median values of PLR in kidney recipients with different types of acute rejection. P values as compared with the corresponding ABMR group; \*  $P < 0.05$ , \*\*  $P < 0.01$ . Figure created using STATISTICA 13.3 (Tibco, Inc., Palo Alto, CA, USA).



**Figure 3.** Pre-transplant and early post-transplant median values of N/LP in kidney recipients with different types of acute rejection. P values as compared with the corresponding ABMR group; \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ . Figure created using STATISTICA 13.3 (Tibco, Inc., Palo Alto, CA, USA).



**Figure 4.** (A-C) The ROC analysis for the values of NLR, PLR, and N/LP ratios calculated at the time of kidney biopsy (Bx), which increased the risk for antibody-mediated rejection. Figure created using STATISTICA 13.3 (Tibco, Inc., Palo Alto, CA, USA).

(72-94%) specificity for NLR, 70% (47-87%) sensitivity and 81% (67-91%) specificity for PLR, and 70% (47-87%) sensitivity and 85% (72-94%) specificity for N/LP (Figure 5A-5C).

Notably, there were significant correlations between NLR, PLR, and N/LP values at both analyzed time-points [Bx: NLR and PLR ( $r=0.813$ ); NLR and N/LP ( $r=0.950$ ); PLR and N/LP ( $r=0.695$ ); Bx-3: NLR and PLR ( $r=0.766$ ); NLR and N/LP ( $r=0.960$ ); PLR and N/LP ( $r=0.652$ ), all  $P<0.001$ ]. Therefore, the creation of any combined parameter for the better prediction of ABMR was not possible.

### Univariate and Multivariate Analyses

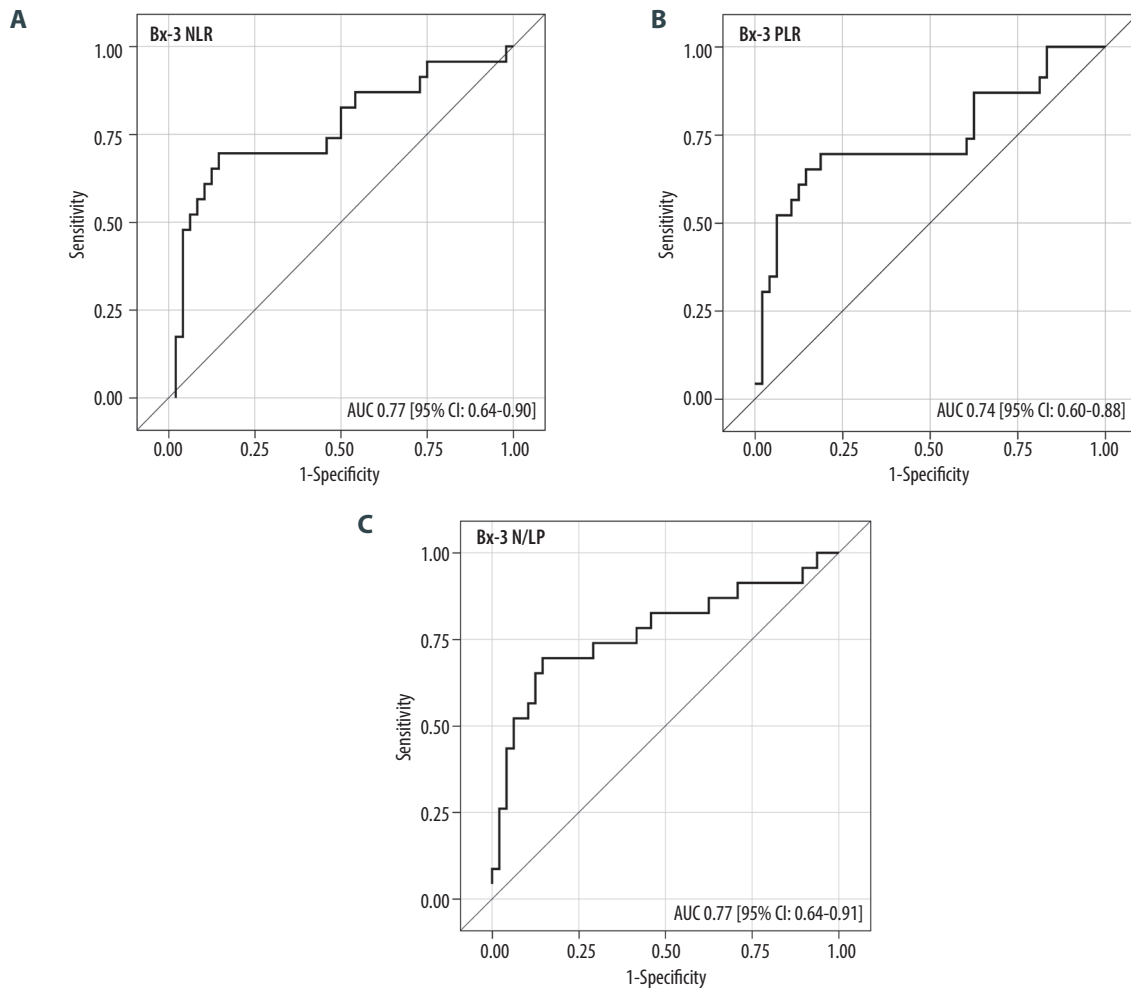
In those analyses, we compared ABMR cases with all no-ABMR cases (TCMR, VR, and rejection-free patients). The results of univariate analyses are shown in Table 3. In multivariate regression models, including CIT, retransplantation, and

different blood count-based ratios, all 3 ratios (NLR, PLR, and N/LP) measured at both Bx and Bx-3 time-point were found to be independently associated with the occurrence of ABMR in protocol biopsy (Table 4). Moreover, CIT was also independently associated with ABMR occurrence in models including NLR, PLR, or N/LP values calculated at the day of the biopsy, but not at the Bx-3 time-point.

### Discussion

In the present study we examined the utility of different inflammatory markers calculated based on the blood cell count in the differential diagnosis of an early post-transplant acute rejection episode in kidney transplant recipients. None of the analyzed markers could distinguish between patients with or without AR in the early post-transplant period. However, we found significantly higher values of NLR, PLR, and N/LP ratios





**Figure 5.** (A-C) The ROC analysis for the values of NLR, PLR, and N/LP ratios calculated at the Bx-3 time-point, which increased the risk for antibody-mediated rejection. Figure created using STATISTICA 13.3 (Tibco, Inc., Palo Alto, CA, USA).

in patients with antibody-mediated rejection as compared to other histologic types.

It is worth to noting that 2 earlier analogic analyses in stable kidney transplant patients yielded conflicting results. Ergin et al [13], in a small study of 22 subjects with AR, noted that  $NLR > 2.5$  was associated with rejection episode, whereas Naranjo et al [14] observed substantially lower NLR and PLR values in AR cases. Importantly, only cellular rejection episodes were analyzed in both studies and kidney graft biopsies were performed “for-cause” in the stable transplant recipients months and years after transplantation. There were neither multivariate analysis nor any clinical characteristics of subjects with or without AR episode in the latter study, while 3 independent analyses performed by Ergin et al, due to the limited sample size, included only uric acid level, steroid dose, and NLR as potential independent variables. Additionally, the type of donor

and median time after transplantation were not analyzed in the multivariate analysis despite between-group differences.

In contrast with these previous studies, in our study all analyzed biopsies were performed within the first 2 weeks post-transplant. There were no significant differences in NLR, PLR, or N/LP between patients with and without early post-transplantation AR. Of note, after the procedure, the values of all analyzed parameters were significantly higher on the 3<sup>rd</sup> day and subsequently subsided. Importantly, in the group of patients with confirmed ABMR episode, this initial increase was significantly more pronounced than in VR and TCMR subjects and the values of all 3 inflammatory ratios decreased only partially until the day of kidney graft biopsy. Consequently, the significant differences in NLR, PLR, and N/LP values between patients with ABMR and other types of rejection or no-rejection were still seen 3 days before and on the day of biopsy.

**Table 3.** The results of univariate analysis for the occurrence of ABMR episode.

Variable	Odds ratio	Lower CL	Upper CL	p
Age	1.000	0.961	1.041	1.00
CIT	1.123	1.029	1.227	0.01
Retransplantation	3.846	1.254	11.796	0.02
ECD status	1.731	0.605	4.955	0.31
HLA mismatch	1.667	0.300	9.271	0.56
Bx NLR	1.028	0.996	1.060	0.09
Bx PLR	1.002	1.000	1.003	0.02
Bx N/LP	1.072	1.015	1.132	0.01
Bx-3 NLR	1.075	1.026	1.127	0.003
Bx-3 PLR	1.003	1.001	1.004	0.004
Bx-3 N/LP	1.128	1.049	1.213	0.001

ABMR – antibody-mediated rejection; CIT – cold ischemia time; ECD – extended criteria donor; HLA – human leukocyte antigen; Bx – the day of biopsy; NLR – neutrophil/lymphocyte ratio; PLR – platelet/lymphocyte ratio; N/LP – neutrophil/lymphocyte and platelet ratio, Bx-3 – 3 days prior to biopsy.

**Table 4.** Results of multiple regression analysis for the occurrence of ABMR episode.

Variable	Bx-3 time-point				Bx time-point			
	OR	Lower	Upper	P	OR	Lower	Upper	P
NLR	1.070	1.020	1.123	0.006	1.030	1.000	1.061	0.048
CIT	1.081	0.981	1.191	0.118	1.110	1.011	1.217	0.028
Retransplant	3.187	0.871	11.665	0.080	2.766	0.808	9.467	0.105
PLR	1.003	1.001	1.005	0.006	1.002	1.000	1.003	0.014
CIT	1.085	0.984	1.196	0.101	1.109	1.010	1.219	0.031
Retransplant	3.227	0.868	12.000	0.080	2.932	0.832	10.341	0.094
N/LP	1.111	1.035	1.193	0.004	1.064	1.011	1.120	0.017
CIT	1.075	0.974	1.186	0.148	1.101	1.002	1.209	0.044
Retransplant	2.582	0.689	9.666	0.159	2.664	0.756	9.379	0.127

CIT – cold ischemia time; NLR – neutrophil/lymphocyte ratio; PLR – platelet/lymphocyte ratio; N/LP – neutrophil/lymphocyte and platelet ratio.

The inflammatory markers analyzed in our study were previously reported to be associated with mortality and morbidity in acute coronary syndrome [17], surgical outcomes in cancer patients [18], and having a prognostic value in gangrenous appendicitis [19]. In patients with chronic kidney disease (CKD), NLR and PLR values were shown to be higher than in healthy controls [20] and were positively correlated with other inflammatory markers [21]. Importantly, NLR was found to be predictive for cardiovascular events and all-cause mortality in CKD subjects [22,23], whereas the evidence concerning its utility as a marker for CKD progression remains inconclusive [24,25].

In hemodialysis patients, NLR may be a good marker of stenosis and restenosis of native and prosthetic arteriovenous fistulas [26]. In KTRs, elevated preoperative NLR was associated with DGF after transplantation [20]. Later after transplant, NLR gradually decreased until it became stable at 3 months post-transplant, but a substantial increase was noted in patients who then developed malignant disease [28].

To date, none of these analyzed ratios have been studied in association with early post-transplant AR. However, Fahim et al investigated the amount and composition of immune cells

within glomeruli and peritubular capillaries (PTC) in cellular and humoral allograft rejection occurring relatively shortly after kidney transplantation [29]. They found that not the absolute number of different immune cells within capillaries, but rather their composition can distinguish humoral from cellular rejection, with inflammatory cells accumulating in glomeruli and PTC, especially in C4d-positive biopsies [29]. Of note, expression of pentraxin-3, an acute-phase reactant produced by a variety of cells at sites of local inflammation, was shown to be higher in rejection biopsies [30]. Moreover, plasma and urinary levels of endocan, a proteoglycan exclusively secreted by vascular endothelium, were significantly higher in ABMR than no-rejection or TCMR rejection subjects [31]. Also, inflammatory lesions in areas of atrophy-fibrosis (i-IFTA), which are associated with increased risk of graft failure, were more often found in indication biopsies with concomitant ABMR, but few with TCMR [32].

All the above-mentioned findings may partly explain our recent observation concerning the greater and more pronounced increase of blood cell count-derived inflammatory ratios in patient with ABMR than in other types of rejection, as well as than in rejection-free patients. As ABMR episodes, especially those that are C4d-negative, are frequently underestimated using conventional criteria [33], and subclinical ABMR had the poorest long-term kidney graft survival as compared with TCMR patients [34], we may speculate that based on the abnormal evolution of analyzed inflammatory markers, the suspicion of subclinical ABMR could be raised earlier after transplantation, resulting in earlier final biopsy-proven diagnosis, more efficient treatment, and better long-term kidney transplantation outcomes. This is especially relevant in patients with good early post-transplant kidney graft function, without any clinical suspicion of AR. Most ABMR episodes during this study emerged despite the previous ATG induction, which was prescribed due to the high pre-transplant measures of immunization. Although the specificity and sensitivity of the analyzed markers in predicting ABMR are lower than for newly developed donor-derived cell-free DNA [35], the possibility to calculate those ratios with no additional costs is still attractive.

Limitations of the present study are its retrospective design and small sample size. Also, the routine protocol biopsy program was not being implemented in our center during the whole study period, which could have caused bias, because

during the approximately 1/5 of study period, only for-cause biopsies were performed and analyzed. However, this is the first study to concurrently investigate the efficacy of NLR, PLR, and N/LP as a potential markers of rejection in the very early period after kidney transplantation, including different types of AR. Additionally, in the effort to improve the quality of our research, we used propensity score matching to create the control group of patients without an AR episode, including factors that potentially influence the blood cell count, and we also analyzed inflammatory ratios: patient age, transplant era, retransplantation, and the type of induction treatment.

## Conclusions

There was no difference in NLR, PLR, and N/LP values between patients with and without AR in the early post-transplant period. As subjects with ABMR presented significantly higher values of analyzed inflammatory markers, the routine assessment of those markers might be of value for identifying patients without clinical suspicion of rejection, who may benefit from early kidney graft biopsy. However, these retrospective data should be confirmed in a prospective study.

## Ethics Approval and Consent to Participate

The study protocol adhered to the Declaration of Helsinki and was approved by the Medical University of Silesia Bioethics Committee (PCN/CBN/0022/KB/164/21). Informed consent was not necessary, as the data analysis did not meet the criteria of a medical experiment.

## Availability of Data and Materials

The datasets generated and/or analyzed during the current study are not publicly available due to limitations of ethical approval involving the patient data and anonymity but are available from the corresponding author on reasonable request.

## Declaration of Figures' Authenticity

All figures submitted have been created by the authors, who confirm that the images are original with no duplication and have not been previously published in whole or in part.

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