INTRODUCTION

Kidney transplantation from a deceased or living related donor is the most desired treatment for patients with end-stage renal disease. Owing to modern immunosuppression (particularly calcineurin inhibitors) as well as improved surgical techniques and postoperative care, the short-term allograft survival has increased. However, long-term outcomes have not improved to an equal extent, which remains a challenge for transplant physicians. A major cause of late graft loss is chronic allograft injury. It is an incompletely understood condition characterized clinically by a gradual deterioration of graft function, often associated with increasing proteinuria and arterial hypertension. The main histological feature of chronic allograft injury is interstitial fibrosis and tubular atrophy (IF/TA), which can be classified as mild, moderate, or severe (grade I, II, or III) according to the Banff grading system. It was found that chronic allograft injury, fibrosis, and atrophy in protocol biopsies are associated with subclinical rejection.

OBJECTIVES

The aim of this study was to assess whether the measurement of urinary concentrations of CCL2 and CXCL10 chemokines can be a valuable noninvasive tool for identifying ongoing pathological processes in a kidney allograft.

PATIENTS AND METHODS

The study included 40 patients who underwent a protocol biopsy within 1-year post kidney transplant. The urinary concentrations of CCL2 and CXCL10 with reference to creatinine in urine were assayed in all patients. On the basis of biopsy results, a study group was selected (n = 25), including patients with a diagnosis of interstitial fibrosis and tubular atrophy grades II to III (n = 16), BK virus (BKV) nephropathy (n = 4), or mild inflammatory lesions fulfilling the criteria for mild rejection processes or borderline lesions (n = 11). Patients with normal biopsy results were included in a control group (n = 15).

RESULTS

The ratio of CCL2 to creatinine (CCL2:Cr) was a significant independent predictor of BKV nephropathy (odds ratio, 1.1; 95% CI, 1.0–1.2; \( P = 0.04 \)). The CXCL10:Cr ratio was not found to be an independent predictor of BKV nephropathy (odds ratio, 1.3; 95% CI, 0.99–1.71; \( P = 0.06 \)).

CONCLUSIONS

The CCL2:Cr and CXCL10:Cr ratios may predict BKV nephropathy. The diagnostic value of CCL2 and CXCL10 in BKV infection should be further evaluated.
The R software was used for... ongoing damaging processes such as IF/TA, BKV nephropathy, or mild inflammatory lesions fulfilling the criteria for mild rejection processes or borderline lesions in the kidney allograft.

**PATIENTS AND METHODS**  
**Patient population**  
The study included 40 patients from our transplant center who underwent a protocol biopsy 12 months after a deceased-donor kidney transplant between 2015 and 2017. On the day of biopsy, patients were in good condition and did not have any signs of acute infection (C-reactive protein levels within a reference range). The biopsy procedure was done under ultrasound guidance, using an 18-gauge biopsy needle. Specimens were assessed according to the Banff classification by one renal pathologist.

On the basis of the biopsy results, the study group (n = 25) was selected, including patients with IF/TA grade II or III (n = 16), BKV nephropathy (n = 4), or mild inflammatory lesions fulfilling the criteria for mild rejection processes or borderline lesions (n = 11). Patients with normal biopsy results were included in a control group (n = 15). Kidney recipients with IF/TA grade I (n = 9) were included in the control group as mild IF/TA representing a natural course of transplant lesions in renal allograft 1-year posttransplant. All patients were of white ethnicity and received tacrolimus, mycophenolate mofetil, and steroids as a maintenance immunosuppressive treatment.

The study was approved by the local ethics committee of the Medical University of Warsaw (Warsaw, Poland). All participants provided written informed consent. The study was conducted in accordance with the Declaration of Helsinki.

**Chemokine analysis**  
In all patients, the urinary concentrations of chemokines CCL2 and CXCL10 were assayed and expressed with reference to creatinine in urine to correct for urinary dilution. Urine samples were collected at the day of biopsy and stored at a temperature of −80°C for future analysis. Chemokines were detected and quantified by an enzyme-linked immunosorbent assay (ELISA), according to the manufacturer’s instructions (R&D Systems, Abingdon, United Kingdom). Samples were tested in duplicate for each patient and the mean value was used for analysis. The intra- and interassay coefficients of variation were 5.9% and 5.9%, respectively, for CCL2 and 3.1% and 9.8%, respectively, for CXCL10. The detection limits for ELISAs were 1.7 pg/ml for CCL2 and 1.67 pg/ml for CXCL10.

**Statistical analysis**  
The R software was used for statistical analysis. Categorical data were described as number (percentage), and continuous data were expressed as mean values with standard deviation or median with quartiles 1 and 3 (Q1–Q3). The χ² test or Fisher exact test was used for categorical variables, and the 2-sample t test or Mann–Whitney test, for continuous variables. The normality of distribution was assessed by...
There was no significant difference between the study and control groups regarding age, sex, immunologic features (first transplantation, HLA antigen mismatch), and blood concentrations of tacrolimus at the time of biopsy. Patients in the study group had significantly higher serum concentrations of creatinine, urinary albumin-to-creatinine ratio, and an IF/TA grade. Estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI creatinine equation, and it was significantly lower in the study group.

The urinary chemokine-to-creatinine ratios in the study and control groups are presented in Table 2. The CCL2:Cr ratio was significantly higher in the study group compared with controls. The CXCL10:Cr ratio was increased only in patients diagnosed with BKV nephropathy.

RESULTS The characteristics of patients are shown in Table 1. In all patients, biopsy was performed 12 months after deceased-donor kidney transplant. There was no significant difference between the study and control groups regarding age, sex, immunologic features (first transplantation, HLA antigen mismatch), and blood concentrations of tacrolimus at the time of biopsy. Patients in the study group had significantly higher serum concentrations of creatinine, urinary albumin-to-creatinine ratio, and an IF/TA grade. Estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI creatinine equation, and it was significantly lower in the study group. The urinary chemokine-to-creatinine ratios in the study and control groups are presented in Table 2. The CCL2:Cr ratio was significantly higher in the study group compared with controls. The CXCL10:Cr ratio was increased only in patients diagnosed with BKV nephropathy.

### Table 1 Characteristics of the study population

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Study group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (n = 25)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at biopsy, y, mean (SD)</td>
<td>47.68 (15.67)</td>
<td>46.25 (16.47)</td>
</tr>
<tr>
<td>Male sex, n (%)</td>
<td>18 (72)</td>
<td>10 (62.5)</td>
</tr>
<tr>
<td>First transplantation, n (%)</td>
<td>21 (84)</td>
<td>13 (81.25)</td>
</tr>
<tr>
<td>HLA antigen mismatch</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total, mean (SD)</td>
<td>3.58 (1.35)</td>
<td>3.44 (1.26)</td>
</tr>
<tr>
<td>A mismatch, mean (SD)</td>
<td>1.25 (0.68)</td>
<td>1.19 (0.66)</td>
</tr>
<tr>
<td>B mismatch, mean (SD)</td>
<td>1.50 (0.66)</td>
<td>1.44 (0.73)</td>
</tr>
<tr>
<td>DR mismatch, mean (SD)</td>
<td>0.83 (0.64)</td>
<td>0.81 (0.54)</td>
</tr>
<tr>
<td>Serum creatinine, mg/dl, mean (SD)</td>
<td>1.78 (0.57)</td>
<td>1.82 (0.62)</td>
</tr>
<tr>
<td>eGFR (CKD-EPI), mg/dl/1.72 m², mean (SD)</td>
<td>45.52 (14.59)</td>
<td>43.81 (14.65)</td>
</tr>
<tr>
<td>Urinary albumin-to-creatinine ratio, mg/g, median (Q1–Q3)</td>
<td>16.29 (8.11–110.60)</td>
<td>23.55 (5.65–111.53)</td>
</tr>
<tr>
<td>Blood tacrolimus levels, ng/ml, mean (SD)</td>
<td>6.73 (2.14)</td>
<td>6.03 (1.74)</td>
</tr>
<tr>
<td>IF/TA grade, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>9 (36)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>II</td>
<td>12 (48)</td>
<td>12 (75)</td>
</tr>
<tr>
<td>III</td>
<td>4 (16)</td>
<td>4 (25)</td>
</tr>
</tbody>
</table>

a Compared with the control group

Abbreviations: BKV, BK virus; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration, eGFR, estimated glomerular filtration rate; HLA, human leukocyte antigen; IF/TA, interstitial fibrosis and tubular atrophy
Correlations between urinary chemokines and renal function parameters and total HLA antigen mismatch in the study population are presented in Table 3. The CCL2:Cr ratio was correlated with serum creatinine and total HLA antigen mismatch, and it was negatively correlated with eGFR. There was no correlation between CCL2 and the urinary albumin-to-creatinine ratio. The CXCL10:Cr ratio was negatively correlated with eGFR, while a positive correlation was noted for the urinary albumin-to-creatinine ratio and total HLA antigen mismatch. No correlation of CXCL10:Cr with serum creatinine concentrations was found. All determined correlations had a weak strength.

To assess the prognostic value of CCL2:Cr and CXCL10:Cr for biopsy findings such as IF/TA grades II to III, BKV nephropathy, or mild inflammatory lesions, a univariate logistic regression analysis was performed (Table 4). The CCL2:Cr ratio proved to be a significant predictor for BKV nephropathy (OR, 1.08; 95% CI, 1.02–1.15; P = 0.02). The CXCL10:Cr ratio was identified as a significant predictor of BKV nephropathy (OR, 1.16; 95% CI, 1.02–1.31; P = 0.02). None of the urinary chemokine-to-creatinine ratios were able to predict IF/TA grades II to III or the development of rejection.

In the second step, a multivariate analysis was performed. It revealed that CCL2:Cr is a significant independent predictor of BKV nephropathy (OR, 1.10; 95% CI, 1.0–1.20; P = 0.04). Moreover, it showed that CXCL10:Cr cannot be considered an independent predictor of BKV nephropathy (OR, 0.99; 95% CI, 0.94–1.05; P = 0.81).
a challenging task for a physician. Excessive immunosuppression may significantly compromise the immune system, leading to, for example, BKV infection, cytomegalovirus infection, or increased risk of malignancy, whereas an insufficient dose of immunosuppressants can trigger organ rejection. The proper management requires a balanced approach, and identification of fast tools for the assessment of the immune system status would improve patient care. The utility of different urinary and serum biomarkers, including chemokines CCL2 and CXCL10, has been broadly analyzed.

In our study, the CCL2:Cr ratio was highly elevated and corresponded with pathological lesions in the kidney graft. It correlated with renal function parameters such as serum creatinine and eGFR levels, but not with albuminuria. A correlation with HLA antigen mismatch was also observed. It can be explained by the fact that more mismatches are associated with more rejection episodes, which demands an aggressive immunosuppressive therapy. This, in turn, may raise the risk of infection and cancer, as the human immune system becomes unstable. It is known that BKV infection significantly increases the mRNA expression of the proinflammatory cytokines such as interleukin 6 or chemokine CCL2. The urinary CCL2:Cr ratio occurs to be a strong predictor of BKV nephropathy, which was previously reported only in one paper.

Recent studies on animal models have indicated that CCL2 is a critical mediator of chronic renal injury. In mice with renovascular hypertension, CCL2 deficiency reduced the number of infiltrating mononuclear cells and expression of other proinflammatory cytokines. Those mice showed less cortical atrophy than wild-type mice. Mao and Wu showed that specific genotype in the promoter region of CCL2 may be a risk factor for chronic kidney disease in Caucasians. We suggest that the role of CCL2 in immunocompromised transplant recipients, especially those with BK infection, should be further studied. In our study, CCL2:Cr was not a predictor of IF/TA grades II to III or mild inflammatory lesions, which is in contrast to data from previous research. It is possible that our study was performed too early and the pathological lesions in kidney allograft were not advanced enough.

In our study, CXCL10:Cr did not differ between the study and control groups. However, it correlated with eGFR, albuminuria, and total HLA antigen mismatch. It was reported that CXCL10:Cr is crucial in the initiation and development of acute rejection accompanying T-cell recruitment. In our research, CXCL10:Cr was not a predictor of lesions associated with a mild inflammatory reaction. It could be explained by the fact that in our patients, those processes developed chronically without an evident production of CXCL10. This could be confirmed by the fact that BKV infection is an acute inflammatory state, and in our study, it was associated with CXCL10:Cr. Similar results were obtained by other investigators.

Using bioinformatic methods, Jia et al revealed that CXCL10 is one of hub proteins in the pathogenesis of BKV nephropathy. It promotes inflammation and inhibits tissue damage repair. Recently, Popik et al showed that BKV may induce CXCL10 in podocytes and mesangial cells, which could contribute to fibrosis in BKV nephropathy and enhance glomerular inflammation.

This study has some major limitations. It was a single-center study on a relatively small study population. The number of patients with BKV nephropathy was low; therefore, no definitive conclusions can be drawn. In conclusion, urinary CCL2:Cr and CXCL10:Cr ratios may be promising predictors of BKV nephropathy, but further studies are needed to confirm that. Both chemokines are measured by an ELISA and they could be easily introduced into routine clinical practice.

ARTICLE INFORMATION

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CONTRIBUTION STATEMENT MSG, MC, and JG analyzed the data. MSG and JG wrote the manuscript. MSG prepared the tables. AS and KC performed experiments. DD-M, MK, and MD were involved in the study design. AF contributed to the writing of the manuscript and assessed the renal biopsy specimens. MD supervised the work and offered expert advice. All authors reviewed and approved the final version of the manuscript.

CONFLICT OF INTEREST None declared.

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REFERENCES

Association of urinary CCL2 and CXCL10 chemokines with BK virus nephropathy


