ORIGINAL ARTICLE

Urinary levels of CCL2 and CXCL10 chemokines as potential biomarkers of ongoing pathological processes in kidney allograft: an association with BK virus nephropathy

Michał S. Gniewkiewicz[1], Magdalena Czerwińska[1,2], Jolanta Gozdowska[1], Katarzyna Czerwińska[1], Anna Sadowska[1], Dominika Dęborska-Materkowska[1], Agnieszka Perkowska-Ptasińska[1], Maciej Kosieradzki[3], Magdalena Durlik[1]

1 Department of Transplantation Medicine, Nephrology and Internal Medicine, Medical University of Warsaw, Warsaw, Poland
2 Laboratory of Centre for Preclinical Research, Department of Experimental and Clinical Physiology, Medical University of Warsaw, Warsaw, Poland
3 Department of General and Transplantation Surgery, Medical University of Warsaw, Warsaw, Poland

KEY WORDS

BK virus nephropathy, CCL2, chemokines, CXCL10, kidney transplantation

ABSTRACT

INTRODUCTION Early prognostic markers that identify high-risk kidney transplant recipients may lead to optimization of immunosuppressive therapy and improved long-term outcomes.

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.

Correspondence to:
Jolanta Gozdowska, MD, PhD, Department of Transplantation Medicine, Nephrology and Internal Medicine, Medical University of Warsaw, ul. Nowogrodzka 59, 02-006 Warszawa, Poland, phone: +48225021232, email: jgozdowska@wum.edu.pl
Received: June 18, 2019.
Revision accepted: August 7, 2019.
Published online: August 7, 2019.
Pol Arch Intern Med. 2019; 129 (9): 592-597
doi:10.20452/pamw.14926

INTRODUCTION Kidney transplantation from a deceased or living related donor is the most desired treatment for patients with end-stage renal disease.[1] Owing to modern immunosuppression (particularly calcineurin inhibitors) as well as improved surgical techniques and postoperative care, the short-term allograft survival has increased.[2] However, long-term outcomes have not improved to an equal extent, which remains a challenge for transplant physicians.[3] A major cause of late graft loss is chronic allograft injury.[4] It is an incompletely understood condition characterized clinically by a gradual deterioration of graft function, often associated with increasing proteinuria and arterial hypertension.[5] 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.[6,7] It was found that chronic allograft injury, fibrosis, and atrophy in protocol biopsies are associated with subclinical rejection.[8]

The other leading cause of premature graft loss is BK virus (BKV) nephropathy, which is characterized by inflammatory interstitial nephropathy.[9] It is associated with the necessity to use higher doses of immunosuppressants, mainly in the first year after transplantation, and it affects up to 10% of kidney graft recipients.[10] There are no antiviral agents with strong evidence for the treatment of BKV infection. The usual strategy on identification of BKV is a gradual reduction of immunosuppression.[11,12]

Currently, renal biopsy is the gold standard in the assessment of kidney function.[13] Protocol biopsies performed at 3 and 12 months postoperatively, as part of standard posttransplant management, are considered to be safe procedures with the risk rate of major complications from 0.33% to 1%.[14,15] They have been applied in this setting because the clinical signs and symptoms of renal allograft dysfunction (such as creatinine increase or proteinuria) manifest relatively late, although an underlying pathology is already advanced.[16] Early detection of active inflammatory processes may lead to optimization of immunosuppressive therapy and improvement of long-term outcomes.[17] Unfortunately, biopsy is an invasive procedure, which is contraindicated in some patients and cannot be performed repetitively. Therefore, monitoring the progression of injury and inflammation is limited.[18] Urinary biomarkers are believed to be promising noninvasive tools useful for an early detection and evaluation of allograft dysfunction.[19] Urine seems to be the most attractive specimen as it is easily accessible and directly indicates the real-time condition of the kidney.[20]

Chemokines CCL2 (also called monocyte chemoattractant protein 1) and CXCL10 (interferon-γ-induced protein of 10 kDa) are small proteins involved in alloimmune response to kidney allograft injury.[21] CCL2 is produced in the kidney by mesangial, tubular epithelial, and infiltrating inflammatory cells and acts by binding to the C-C chemokine receptor 2.[22,23] It plays an important role in chemoattraction of monocytes, T lymphocytes, and natural killer cells, and also in generation and function of memory $CD8^+$ T cells.[24,25] CXCL10 is secreted in response to interferon γ by a variety of cells, including leukocytes and kidney mesangial cells.[26] It binds to the CXCR3 receptor that is located on the immune cells, especially T cells, which results in their recruitment into the allograft and amplification of immune reaction.[27] CXCL10 seems to be crucial in the immune response to the transplanted organ. As shown by Hancock et al,[28] CXCR3−/− mice, in comparison with CXCR3+/+ mice, present a delay or even an absence of acute or chronic rejection of cardiac graft, and that anti-CXCR3 monoclonal antibody is able to stop the development of the transplant rejection.[28]

The aim of this prospective study was to assess whether the measurement of urinary concentrations of chemokines CCL2 and CXCL10 can be a valuable noninvasive tool for identifying 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.[7]

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.[29,30] 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.[31] 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 χ^2 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

TABLE 1 Characteristics of the study population

Parameter	Study group				Control group (n = 15)
	Total (n = 25)	IF/TA grade II–III (n = 16)	BKV nephropathy (n = 4)	Mild inflammatory lesions (n = 11)	
Age at biopsy, y, mean (SD)	47.68 (15.67)	46.25 (16.47)	50.50 (17.1)	45.27 (17.56)	50.15 (11.95)
P value[a]	0.43	0.31	0.89	0.29	–
Male sex, n (%)	18 (72)	10 (62.5)	4 (100)	8 (72.73)	10 (66.67)
P value[a]	0.5	1	0.26	0.69	–
First transplantation, n (%)	21 (84)	13 (81.25)	3 (75)	10 (90.91)	14 (93.33)
P value[a]	0.63	0.6	0.39	1	–
HLA antigen mismatch					
Total, mean (SD)	3.58 (1.35)	3.44 (1.26)	3.75 (1.5)	3.5 (1.58)	3 (1.15)
P value[a]	0.23	0.3	0.42	0.49	–
A mismatch, mean (SD)	1.25 (0.68)	1.19 (0.66)	1.25 (0.96)	1.2 (0.63)	1.1 (0.74)
P value[a]	0.59	0.79	0.76	0.8	–
B mismatch, mean (SD)	1.50 (0.66)	1.44 (0.73)	1.5 (0.6)	1.3 (0.83)	1.1 (0.57)
P value[a]	0.07	0.16	0.28	0.46	–
DR mismatch, mean (SD)	0.83 (0.64)	0.81 (0.54)	1 (0.82)	1 (0.67)	0.7 (0.67)
P value[a]	0.58	0.57	0.96	0.9	–
Serum creatinine, mg/dl, mean (SD)	1.78 (0.57)	1.82 (0.62)	2.21 (0.5)	1.62 (0.47)	1.32 (0.19)
P value[a]	0.005	0.006	<0.001	0.04	–
eGFR (CKD-EPI), mg/dl/1.72 m^2, mean (SD)	45.52 (14.59)	43.81 (14.65)	37.75 (13.18)	46.68 (11.98)	58.2 (13.07)
P value[a]	0.009	0.007	0.01	0.04	–
Urinary albumin-to-creatinine ratio, mg/g, median (Q1–Q3)	16.29 (8.11–110.60)	23.55 (5.65–111.53)	37.58 (15.72–59.43)	74.81 (8.69–253.69)	6.28 (2.58–15.13)
P value[a]	0.04	0.03	0.03	0.02	–
Blood tacrolimus levels, ng/ml, mean (SD)	6.73 (2.14)	6.03 (1.74)	7.35 (1.48)	6.72 (1.66)	6.91 (2.37)
P value[a]	0.82	0.25	0.80	0.83	–
IF/TA grade, %					
I	9 (36)	0 (0)	4 (100)	5 (45.4)	9 (15)
II	12 (48)	12 (75)	0 (0)	5 (45.5)	0 (0)
III	4 (16)	4 (25)	0 (0)	1 (9.09)	0 (0)
P value	<0.001	<0.001	0.5	0.01	–

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

the Shapiro–Wilk test. Correlations were determined using the Spearman rank correlation coefficient. The predictive value of the ratio of urinary chemokine to creatinine for the occurrence of pathological features in biopsy results (IF/TA grade II or III, BKV nephropathy, mild inflammatory lesions) was tested using univariate and multivariate logistic regression models. The ratios of CCL2 to creatinine (CCL2:Cr) and of CXCL10 to creatinine (CXCL10:Cr) were included as covariates in the multivariate model. Odds ratios (ORs) with 95% CIs were calculated. *P* values of less than 0.05 were considered significant.

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 2 Urinary chemokine-to-creatinine ratios in the study population

Parameter	Study group				Control group (n = 15)
	Total (n = 25)	IF/TA grade II–III (n = 16)	BKV nephropathy (n = 4)	Mild inflammatory lesions (n = 11)	
CCL2:Cr, ng/mmol, median (Q1–Q3)	17.21 (8.32–30.81)	17.82 (8.32–27.55)	39.76 (25.43–61.56)	10.68 (9.03–17.33)	7.44 (6.17–9.23)
P value[a]	0.004	0.01	0.001	0.02	–
CXCL10:Cr, ng/mmol, median (Q1–Q3)	5.75 (2.15–8.92)	3.53 (2.15–7.63)	22 (9.25–34.75)	4.29 (2.74–7.64)	3.87 (1.21–8.9)
P value[a]	0.31	0.68	0.03	0.61	–

a Compared with the control group

Abbreviations: CCL2:Cr, ratio of chemokine CCL2 to creatinine; CXCL10:Cr, ratio of chemokine CXCL10 to creatinine; others, see TABLE 1

TABLE 3 Correlation between urinary chemokine-to-creatinine ratios and renal function and total HLA antigen mismatch

Parameter	Urinary CCL2:Cr		Urinary CXCL10:Cr	
	Correlation coefficient	*P* value	Correlation coefficient	*P* value
Serum creatinine	0.38	0.02	0.25	0.11
eGFR (CKD-EPI)	–0.35	0.03	–0.32	0.04
Urinary albumin-to-creatinine ratio	0.23	0.28	0.42	0.04
Total HLA antigen mismatch	0.36	0.04	0.47	0.005

Abbreviations: see TABLES 1 and 2

TABLE 4 Univariate and multivariate logistic regression analysis

Variable	IF/TA II–III				BKV nephropathy				Mild inflammatory lesions			
	Univariate		Multivariate[a]		Univariate		Multivariate[a]		Univariate		Multivariate[a]	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
CCL2:Cr	1.02 (0.98–1.06)	0.35	1.02 (0.98–1.07)	0.31	1.08 (1.02–1.15)	0.02	1.10 (1–1.20)	0.04	0.99 (0.94–1.05)	0.81	0.99 (0.93–1.04)	0.65
CXCL10:Cr	0.99 (0.92–1.08)	0.9	0.98 (0.89–1.07)	0.98	1.16 (1.02–1.31)	0.02	1.3 (0.99–1.71)	0.06	0.93 (0.81–1.07)	0.34	0.97 (0.84–1.11)	0.63

a Multivariate logistic regression analysis includes CCL2:Cr and CXCL10:Cr as covariates.

Abbreviations: OR, odds ratio; others, see TABLES 1 and 2

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, 1.1; 95% CI, 1.0–1.2; $P = 0.04$). Moreover, it showed that CXCL10:Cr cannot be considered an independent predictor of BKV nephropathy (OR, 1.3; 95% CI, 0.99–1.71; $P = 0.06$).

DISCUSSION The management of immunosuppression in a kidney transplant recipient is

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.[32] 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.[33,34]

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.[35] It is known that BKV infection significantly increases the mRNA expression of the proinflammatory cytokines such as interleukin 6 or chemokine CCL2.[36] The urinary CCL2:Cr ratio occurs to be a strong predictor of BKV nephropathy, which was previously reported only in one paper.[37]

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.[38,39] Mao and Wu[40] 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.[41,42] 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.[43,44] 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.[45,46] Using bioinformatic methods, Jia et al[47] revealed that CXCL10 is one of hub proteins in the pathogenesis of BKV nephropathy. It promotes inflammation and inhibits tissue damage repair.[47] Recently, Popik et al[48] 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

ACKNOWLEDGMENTS This work was supported by a grant from Ministry of Science and Higher Education of Poland (grant number: MNiSW/2017/112/DIR/NN2; to MSG, MC, and JG).

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. AP-P 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.

HOW TO CITE Gniewkiewicz MS, Czerwińska M, Gozdowska J, et al. Urinary levels of CCL2 and CXCL10 chemokines as potential biomarkers of ongoing pathological processes in kidney allograft: an association with BK virus nephropathy. Pol Arch Intern Med. 2019; 129: 592-597. doi:10.20452/pamw.14926

REFERENCES

1 Tonelli M, Wiebe N, Knoll G, et al. Systematic review: kidney transplantation compared with dialysis in clinically relevant outcomes. Am J Transplant. 2011; 11: 2093-2109.

2 Tantravahi J, Womer KL, Kaplan B. Why hasn't eliminating acute rejection improved graft survival? Annu Rev Med. 2007; 58: 369-385.

3 Lamb KE, Lodhi S, Meier-Kriesche HU. Long-term renal allograft survival in the United States: a critical reappraisal. Am J Transplant. 2011; 11: 450-462.

4 Jevnikar AM, Mannon RB. Late kidney allograft loss: what we know about it, and what we can do about it. Clin J Am Soc Nephrol. 2008; 3 Suppl 2: S56-S67.

5 Pascual J, Pérez-Sáez MJ, Mir M, Crespo M. Chronic renal allograft injury: early detection, accurate diagnosis and management. Transplant Rev (Orlando). 2012; 26: 280-290.

6 Li L, Greene I, Readhead B, et al. Novel therapeutics identification for fibrosis in renal allograft using integrative informatics approach. Sci Rep. 2017; 7: 39487.

7 Haas M. The revised (2013) Banff classification for antibody-mediated rejection of renal allografts: update, difficulties, and future considerations. Am J Transplant. 2016; 16: 1352-1357.

8 Moreso F, Ibernon M, Gomà M, et al. Subclinical rejection associated with chronic allograft nephropathy in protocol biopsies as a risk factor for late graft loss. Am J Transplant. 2006; 6: 747-752.

9 Jia L, Fu W, Jia R, et al. Identification of potential key protein interaction networks of BK virus nephropathy in patients receiving kidney transplantation. Sci Rep. 2018; 8: 5017.

10 Ribeiro A, Wörnle M, Motamedi N, et al. Activation of innate immune defense mechanisms contributes to polyomavirus BK-associated nephropathy. Kidney Int. 2012; 81: 100-111.

11 Ambalathingal GR, Francis RS, Smyth MJ, et al. BK polyomavirus: clinical aspects, immune regulation, and emerging therapies. Clin Microbiol Rev. 2017; 30: 503-528.

12 Dalianis T, Eriksson BM, Felldin M, et al. Management of BK-virus infection - Swedish recommendations. Infect Dis (Lond). 2019; 51: 479-484.

13 Henderson LK, Nankivell BJ, Chapman JR. Surveillance protocol kidney transplant biopsies: their evolving role in clinical practice. Am J Transplant. 2011; 11: 1570-1575.

14 Morgan TA, Chandran S, Burger IM, et al. Complications of ultrasound--guided renal transplant biopsies. Am J Transplant. 2016; 16: 1298-1305.

15 Schwarz A, Gwinner W, Hiss M, et al. Safety and adequacy of renal transplant protocol biopsies. Am J Transplant. 2005; 5: 1992-1996.

16 Huang Y, Farkash E. Protocol Biopsies: Utility and Limitations. Adv Chronic Kidney Dis. 2016; 23: 326-331.

17 Wilkinson A. Protocol transplant biopsies: are they really needed? Clin J Am Soc Nephrol. 2006; 1: 130-137.

18 Merhi B, Bayliss G, Gohh RY. Role for urinary biomarkers in diagnosis of acute rejection in the transplanted kidney. World J Transplant. 2015; 5: 251-260.

19 Quintana LF, Solé-Gonzalez A, Kalko SG, et al. Urine proteomics to detect biomarkers for chronic allograft dysfunction. J Am Soc Nephrol. 2009; 20: 428-435.

20 Salcido-Ochoa F, Allen JC. Biomarkers and a tailored approach for immune monitoring in kidney transplantation. World J Transplant. 2017; 7: 276-284.

21 Elmoselhi H, Mansell H, Soliman M, Shoker A. Circulating chemokine ligand levels before and after successful kidney transplantation. J Inflamm (Lond). 2016; 13: 32.

22 Segerer S, Nelson PJ, Schlöndorff D. Chemokines, chemokine receptors, and renal disease: from basic science to pathophysiologic and therapeutic studies. J Am Soc Nephrol. 2000; 11: 152-176.

23 Moyses neto M, Romão EA, Silva GE, et al. Urinary monocyte chemoattractant protein-1 levels and interstitial changes in the renal cortex and their relationship with loss of renal function in renal transplant patients with delayed graft function. Can J Kidney Health Dis. 2015; 2: 1.

24 Raza A, Firasat S, Khaliq S, et al. Monocyte chemoattractant protein-1 (MCP-1/CCL2) levels and its association with renal allograft rejection. Immunol Invest. 2017; 46: 251-262.

25 Wang T, Dai H, Wan N, et al. The role for monocyte chemoattractant protein-1 in the generation and function of memory CD8+ T cells. J Immunol. 2008; 180: 2886-2893.

26 Gao J, Wu L, Wang Y, et al. Knockdown of Cxcl10 inhibits mesangial cell proliferation in murine Habu nephritis via ERK signaling. Cell Physiol Biochem. 2017; 42: 2118-2129.

27 Lo DJ, Kaplan B, Kirk AD. Biomarkers for kidney transplant rejection. Nat Rev Nephrol. 2014; 10: 215-225.

28 Hancock WW, Lu B, Gao W, et al. Requirement of the chemokine receptor CXCR3 for acute allograft rejection. J Exp Med. 2000; 192: 1515-1520.

29 Stegall MD, Park WD, Larson TS, et al. The histology of solitary renal allografts at 1 and 5 years after transplantation. Am J Transplant. 2011; 11: 698-707.

30 Mengel M, Chang J, Kayser D, et al. The molecular phenotype of 6-week protocol biopsies from human renal allografts: reflections of prior injury but not future course. Am J Transplant. 2011; 11: 708-718.

31 Tang KW, Toh QC, Teo BW. Normalisation of urinary biomarkers to creatinine for clinical practice and research - when and why. Singapore Med J. 2015; 56: 7-10.

32 Kalluri HV, Hardinger KL. Current state of renal transplant immunosuppression: present and future. World J Transplant. 2012; 2: 51-68.

33 Erpicum P, Hanssen O, Weekers L, et al. Non-invasive approaches in the diagnosis of acute rejection in kidney transplant recipients, part II: omics analyses of urine and blood samples. Clin Kidney J. 2017; 10: 106-115.

34 Litman-Zawadzka A, Łukaszewicz-Zając M, Gryko M, et al. Serum chemokine CXCL8 as a better biomarker for diagnosis and prediction of pancreatic cancer than its specific receptor CXCR2, C-reactive protein, and classic tumor markers CA 19-9 and CEA. Pol Arch Intern Med. 2018; 128: 524-531.

35 Zachary AA, Leffell MS. HLA Mismatching strategies for solid organ transplantation - a balancing act. Front Immunol. 2016; 7: 575.

36 Ribeiro A, Wörnle M, Motamedi N, et al. Activation of innate immune defense mechanisms contributes to polyomavirus BK-associated nephropathy. Kidney Int. 2012; 81: 100-111.

37 Boratyńska M, Dubiński B, Rybka K, et al. Immunocytological urinalysis and monocyte chemotactic peptide-1 in renal transplant recipients with polyomavirus replication. Transplant Proc. 2006; 38: 151-154.

38 Kashyap S, Osman M, Ferguson CM, et al. Ccl2 deficiency protects against chronic renal injury in murine renovascular hypertension. Sci Rep. 2018; 8: 8598.

39 Lv LL, Feng Y, Wen Y, et al. Exosomal CCL2 from tubular epithelial cells is critical for albumin-induced tubulointerstitial inflammation. J Am Soc Nephrol. 2018; 29: 919-935.

40 Mao S, Wu L. Association between MCP-1 2518 A>G gene polymorphism and chronic kidney disease. Int Urol Nephrol. 2018; 50: 2245-2253.

41 Ho J, Rush DN, Gibson IW, et al. Early urinary CCL2 is associated with the later development of interstitial fibrosis and tubular atrophy in renal allografts. Transplantation. 2010; 90: 394-400.

42 Hirt-Minkowski P, Rush DN, Gao A, et al. Six-month urinary CCL2 and CXCL10 levels predict long-term renal allograft outcome. Transplantation. 2016; 100: 1988-1996.

43 Rosenblum JM, Shimoda N, Schenk AD, et al. CXC chemokine ligand (CXCL) 9 and CXCL10 are antagonistic costimulation molecules during the priming of alloreactive T cell effectors. J Immunol. 2010; 184: 3450-3460.

44 Merhi B, Bayliss G, Gohh RY. Role for urinary biomarkers in diagnosis of acute rejection in the transplanted kidney. World J Transplant. 2015; 5: 251-260.

45 Jackson JA, Kim EJ, Begley B, et al. Urinary chemokines CXCL9 and CXCL10 are non-invasive markers of renal allograft rejection and BK viral infection. Am J Transplant. 2011; 11: 2228-2234.

46 Hu H, Aizenstein BD, Puchalski A, et al. Elevation of CXCR3-binding chemokines in urine indicates acute renal-allograft dysfunction. Am J Transplant. 2004; 4: 432-437.

47 Jia L, Fu W, Jia R, et al. Identification of potential key protein interaction networks of BK virus nephropathy in patients receiving kidney transplantation. Sci Rep. 2018; 8: 5017.

48 Popik W, Khatua AK, Fabre NF, et al. BK virus replication in the glomerular vascular unit: implications for BK virus associated nephropathy. Viruses. 2019; 11.