Longitudinal assessment of the CXCL10 blood and urine concentration in kidney transplant recipients with BK polyomavirus replication—a retrospective study

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SUMMARY
In kidney transplant recipients (KTRs), BK polyomavirus (BKPyV) replication may progress to polyomavirus-associated nephropathy (PVAN). In this retrospective study, we assessed the chemokine CXCL10 in urine and blood samples consecutively acquired from 85 KTRs who displayed different stages of BKPyV replication and eventually developed PVAN. In parallel to progression toward PVAN, CXCL10 gradually increased in blood and urine, from baseline (prior to virus replication) to BKPyV DNAuria (median increase in blood: 42.15 pg/ml, \(P = 0.0156\)), from mere DNAuria to low- and high-level BKPyV DNAemia (median increase: 52.60 and 87.26 pg/ml, \(P = 0.0010\) and \(P = 0.0002\), respectively) and peaked with histologically confirmed PVAN (median increase: 145.00 pg/ml, \(P < 0.0001\)). CXCL10 blood and urine levels significantly differed among KTRs with respect to simultaneous presence of human cytomegalovirus (\(P < 0.001\)) as well as in relation to the clinical severity of respective BKPyV DNAemia episodes (\(P = 0.0195\)). CXCL-10 concentrations were particularly lower in KTRs in whom BKPyV DNAemia remained without clinical evidence for PVAN, as compared to individuals who displayed high decoy cell levels, decreased renal function and/or biopsy-proven PVAN (median blood concentration: 266.97 vs. 426.42 pg/ml, \(P = 0.0282\)). In conclusion, in KTRs CXCL10 rises in parallel to BKPyV replication and correlates with the gradual development of PVAN.

Key words
BK virus, CXCL10, kidney transplantation, nephropathy, polyomavirus

Introduction
BK virus (BKPyV) and JC virus (JCPyV), two human polyomaviruses, cause persistent infection, with the urinary tract as most relevant latency site [1]. Since cellular immunity plays a key role in control of these viruses, immunosuppressive treatment after kidney transplantation (KTX) may lead to their reactivation, usually first indicated by asymptomatic viruria [2–8]. Especially when viruria is sustained in the long term, it may be followed by viremia, and progression of viral replication is subsequently indicated by occurrence of high viral
loads in urine and blood, which may finally result in significant inflammation, parenchymal injury, and symptomatic disease, termed as polyomavirus-associated nephropathy (PVAN) [2–4,9–12].

Although BKPyV is the main causative agent, JCPyV may also trigger PVAN [5,13]. Quantitative measurement of BKPyV with or without JCPyV DNA in urine and blood is therefore commonly undertaken during the post-transplant surveillance [2,3,14,15]. Additional correlates of clinical progression are emergence of decoy cells in the urinary sediment, an indicator for cytopathic effects due to urothelial cell infection, the detection of viral aggregates by the Haufen test, BKPyV VP1 mRNA as well as histological evidence of viral inclusions, tubular injury, and inflammatory infiltrates [2–4,16–18].

Host factors, as well as viral determinants, account for the complex pathogenesis of PVAN [3,4]. Immune responses, which on one hand, are essential to control viral replication may on the other hand mediate renal injury. In this process, like in all inflammatory processes, chemokines, structurally related, small chemotactic cytokines orchestrate cell–cell signaling and immune cell trafficking [19,20]. Since chemokines shape immune responses against specific pathogens by recruiting particular subsets of leukocytes to the infection site, they play an essential role and may be used as inflammatory biomarkers in viral diseases [21–23].

In this study, we longitudinally assessed levels of CXC-ligand 10 (CXCL10), a chemokine which attracts and activates immune cells with a cytokine profile of T-helper cells Type 1 (Th1), aiming to determine whether CXCL10 correlates with an increase and clinical progression of BKPyV replication [24–26]. Our analysis also included the chemokines CCL8, CXCL16, and CCL20, candidate markers for outcome and severity of human cytomegalovirus (HCMV) infections in solid organ recipients [22,23,27].

Materials and methods

Patients and sample acquisition

This retrospective study included pairs of blood and urine samples from a total of 95 KTRs (35 female, 60 male) including controls, who underwent KTX between March 2008 and September 2014. All KTRs underwent virological routine testing which consisted of PCR analyses for BKPyV and JCPyV DNA in urine and blood, collected pairwise on the same day of the post-transplant follow-up, respectively, and HCMV DNA in blood. Eighty-five patients were selected for the study by retrospective review of virological test results and were included when at least one episode of BKPyV DNAemia with DNA loads ≥1000 copies/ml occurred during the post-transplant follow-up. Ten KTRs, who underwent transplantation and post-transplant surveillance during the same time period, served as controls since they lacked any evidence of BKPyV, JCPyV, or HCMV replication (see Table S1). Further information on the immunosuppressive treatment, the schedule of sample acquisition and virological routine testing as well as the storage of samples is given in Supplemental Material and Methods. Sample collection and storage as well as the whole study protocol was approved by the local ethics committee of Medical University of Vienna, which concluded that no written informed consent from the patients was required (EK1035/2016, EK2064/2016). The study was performed in accordance with the Helsinki Declaration.

Quantitative PCR

Information on the protocols used for quantitative BKPyV, JCPyV, and HCMV PCR analyses is given in Appendix S1.

Criteria for grouping patients and selection of samples

Out of the 85 KTRs, who were included in the study because they displayed at least one episode of BKPyV DNAemia, 56 displayed BKPyV DNAemia in the absence of significant JCPyV and HCMV DNAemia. Thirteen KTRs simultaneously developed BKPyV and JCPyV DNAemia (>1000 copies/ml, respectively) and 16 simultaneously developed BKPyV and HCMV DNAemia. Among these groups, there was no significant difference with respect to gender, age, transplant type, donor characteristics, cold ischemia time, delayed graft function, HLA-mismatches, AB0 and HLA-incompatibilities, duration of follow-up and the median time point BKPyV DNAemia occurred (Table S1).

To assess individual CXCL-10 kinetics during progression of BKPyV replication, we retrieved and analyzed 148 specific blood/urine sample pairs, collected from the 56 KTRs with mere BKPyV HCMV DNAemia due to routine testing. These samples specifically fulfilled the inclusion requirement that they had been collected during different stages of BKPyV replication (mean number of sample pairs per patient: 2.64, range: 2–4). As shown in Table 1, these stages were defined as: (i) baseline (before BKPyV detection, with undetectable
BKPyV, JCPyV, and HCMV DNA in urine and blood, no evidence for any other infection nor rejection, normal levels of C-reactive protein and leukocytes, normal urinary cytology, no drop in renal function), (ii) BKPyV detection only in urine (BKPyV DNAuria) but not in blood, (iii) BKPyV DNAuria plus low-level BKPyV DNAemia (blood DNA levels <1000 copies/ml), (iv) BKPyV DNAuria plus BKPyV DNAemia (blood DNA levels ≥1000 copies/ml) but no significant decoy cell levels, (v) BKPyV DNAemia (>1000 copies/ml) together with detection of decoy cell levels >20%, a decrease of eGFR ± histological evidence for PVAN. The mean sampling intervals are shown in Table 1. When multiple samples were acquired from one patient during the same stage of BKPyV replication, only the initial sample that fulfilled the respective criteria was selected for the study.

In order to compare chemokine levels among individual patients, the 56 KTRs with mere BKPyV DNAemia were sub-grouped based on the clinical severity of the respective BKPyV DNAemia episode. Although their BKPyV DNA loads exceeded 1000 copies/ml, 9 of the 56 KTRs displayed no significant decoy cell levels (<5%) in urinary sediment during any BKPyV DNAemia episode, renal function remained stable and no biopsy was therefore performed during the entire follow-up. The other 47 KTRs, in contrast, displayed at least one BKPyV DNAemia episode in which significant decoy cell levels (defined as >20%) were concomitantly detected. Of those 47, 23 (48.9%) additionally showed a ≥15% decrease of estimated glomerular filtration rate (eGFR), as compared to the mean of three measurements which immediately preceded the initial occurrence of the respective high-level DNAemia episode (median interval: 41 days, range: 13–98). In 15 patients, PVAN was confirmed with a renal biopsy obtained within a week before or after blood/urine sampling. In the remaining KTRs, no biopsies were performed within this time period.

In addition to the 47 patients with mere BKPyV DNAemia, all 13 KTRs, who simultaneously displayed high-level BKPyV and JCPyV DNAemia, showed decoy cells >20%. An eGFR decrease occurred in 6 (46.2%) and PVAN was histologically confirmed in 5 of these 13 KTRs.

Histological confirmation of PVAN was based on the detection of injured tubules with or without interstitial nephritis, viral inclusion bodies, and/or positive SV40 T-Antigen staining [28]. In all biopsies with confirmed PVAN, pathologists specifically described no histological evidence for rejection [29]. However, 10 KTRs with high-level BKPyV DNAemia subsequently developed allograft rejection during the same BKPyV DNAemia episode, which was confirmed by histology using Banff criteria (borderline T-cell-mediated rejection: \( n = 7 \), CD4+ rejection: \( n = 1 \), acute cellular rejection: \( n = 1 \), T-cell-mediated rejection \( 2a: n = 1 \)) [29].

As described, urine/blood sample pairs from 10 KTRs who did not display any BKPyV, JCPyV, and HCMV replication were included as controls, and samples from these controls fulfilled the same criteria as baseline samples (C-reactive protein and leukocytes in normal range, normal urinary cytology, and no eGFR decrease).

Quantification of CXCL10, CCL8, CXCL16, and CCL20

CXCL10, CCL8, CXCL16, and CCL20 were quantified by a commercially available ELISA test and LuminexTM xMAP technology [30]. More information is given in Appendix S1.

Statistical methods

Within-patient comparisons of CXCL-10 in blood and urine were performed using paired nonparametric t-tests (Wilcoxon matched-pairs signed-rank test). Differences in CXCL10, CCL8, CCL20, and CXCL16 concentrations (including blood/urine ratios) between KTRs with BKPyV DNAemia with and without progression to PVAN as well as among patients with additional JCPyV, HCMV DNAemia were compared by unpaired nonparametric Kruskal–Wallis and Dunn’s multiple comparison tests. CXCL10 between baseline samples and control individuals was compared by Mann–Whitney t-test. Effect sizes are reported as \( d_{\text{Cohen}} \). Receiver operating characteristic (ROC) analyses were performed to analyze the association of CXCL10 with high-level BKPyV DNAemia, clinical manifestations, and/or histological evidence of PVAN. For all statistical tests, a two-tailed \( P \)-value of <0.05 was considered statistically significant. GRAPHPAD PRISM version 8.0 software was used for statistical analyses.

Results

CXCL10 in blood and urine during progression of BKPyV replication

First, we analyzed whether progression of BKPyV replication toward DNAemia and PVAN was associated with a response of the inflammatory chemokine CXCL10.
Table 1. Criteria for grouping patients and samples acquired during progression of BKPyV replication

| Patient group | BKPyV DNAemia | BKPyV & JCPyV DNAemia | BKPyV & HCMV DNAemia | Controls |
|---------------|---------------|------------------------|----------------------|----------|
| Number of patients (n) | 56 | 13 | 16 | 10 |
| Stages of BKPyV replication | | | | |
| Baseline BKPyV DNAuria, no DNAemia | | | | |
| BKPyV DNAemia (≥10^3 copies/ml) | | | | |
| Decoy cells <20% | | | | |
| BKPyV In urine* | Undetectable | 1.8 x 10^6, 2.7 x 10^6–1.5 x 10^7 | 1.1 x 10^6, 5.5 x 10^5–8.1 x 10^5 | 2.5 x 10^6, 2.6 x 10^6–5.4 x 10^10 |
| In blood* | Undetectable | 4.5 x 10^6, 1.0 x 10^7–9.6 x 10^6 | 4.4 x 10^6, 1.0 x 10^7–1.9 x 10^7 | 1.7 x 10^7–7.4 x 10^9 |
| JCPyV In blood* | Undetectable | Undetectable: 28/31 | Undetectable: 40/47 | Detectable: 7/47 |
| HCMV in blood* | Undetectable | Undetectable | Undetectable | Undetectable |
| Decoy cells in urinary sediment (>20%) | | | | |
| No | No | No | No | No |
| Decrease of eGFR† | No | No | No | No |
| Yes | No | No | No | No |
| PVAN verified by histology‡ | No | No | No | No |
| Number of urine/serum sample pairs§ | 16 | 33 | 21 | 47 |
| Interval between sample acquisition¶ | 70, 20–265 | 184, 56–512 | 177, 56–770 | 136, 41–581 |

BKPyV, BK Polyomavirus; JCPyV, JC Polyomavirus; HCMV, Human Cytomegalovirus; eGFR, estimated glomerular filtration rate; PVAN, Polyomavirus associated nephropathy.

*Median DNA load, range; copies/ml.
†≥15% decrease of eGFR, as compared to mean of the 3 preceding measurements.
§Biopsy within 1 week before/after sample acquisition.
¶One pair per patient and stage respectively.
†Median days, range.
Therefore, we quantified CXCL10 in 148 pairs of urine and blood samples acquired from 56 KTRs with BKPyV (but not JCPyV or HCMV) DNAemia at multiple time points and during different stages of BKPyV replication (Table 1).

As shown in Fig. 1a and Table S2, from first BKPyV detection in urine toward histologically proven PVAN, we observed a gradual increase of CXCL10 in blood. CXCL10 concentrations significantly increased from baseline to BKPyV DNAuria only (median increase: 42.15 pg/ml, 95% CI: 9.11–151.84, $P = 0.016$). This CXCL10 baseline concentration (before BKPyV detection) did not significantly differ from the one in controls who did not display any BKPyV, JCPyV, or HCMV detection during follow-up (median difference: −0.81 pg/ml, 95% CI: −32.72 to 21.98, $P = 0.74$).

CXCL10 in blood further rose when in addition to mere BKPyV DNAuria low-level DNAemia emerged (median increase: 52.60 pg/ml, 95% CI: 41.55–78.82, $P = 0.001$) and further increased when low-level DNAemia progressed to DNA loads ≥1000 copies/ml, but no evidence for PVAN was yet detected (decoy cells <20%, no eGFR decrease; median increase: 87.26 pg/ml, 95% CI: 55.42–153.51, $P = 0.0002$). CXCL10 in blood finally peaked when BKPyV DNAuria and DNAemia concurrently occurred with clinical manifestations (significant decoy cell levels ± eGFR decrease) and/or histological evidence of PVAN (median increase: 145.00 pg/ml, 95% CI: 120.20–227.83, $P < 0.0001$).

Next, we analyzed whether progression of BKPyV replication toward DNAemia and PVAN was associated with an inflammatory CXCL10 response in urine analogously to blood concentrations. Therefore, we quantified CXCL10 in urine samples obtained pairwise with blood samples and found that CXCL10 urine concentrations also increased due to progression of BKPyV replication. As shown in Fig. 1b and Table S2, CXCL10 in urine rose from baseline to BKPyV DNAuria alone (median increase: 14.15 pg/ml, 95% CI: −1.48 to 38.23, $P = 0.0223$) and increased from this to low-level DNAemia (median increase: 36.44 pg/ml, 95% CI: 16.59–80.91, $P = 0.001$). The CXCL10 urine concentration further increased from low-level BKPyV DNAemia when viral DNA loads exceeded 1000 copies/ml but no clinical manifestations of PVAN were observed yet (median increase: 87.59 pg/ml, 95% CI: 39.02–148.07, $P = 0.0005$). CXCL10 in urine finally peaked when, in addition to DNAemia, significant levels of decoy cells, a decrease of eGFR and/or histological evidence for PVAN were detected (median increase: 173.50 pg/ml, 95% CI: 129.27–280.41, $P < 0.0001$).

The individual blood/urine ratio of the CXCL10 concentration did not significantly change during different stages of BKPyV replication until an eGFR decrease occurred and/or biopsy-proven PVAN developed. As compared to the other stages, evidence for PVAN was associated with a significant decrease of the ratio due to a disproportionately strong increase of urine CXCL10 levels (median change of the blood/urine ratio: −0.89, 95% CI: −7.38 to −0.69, $P = 0.0042$).

CXCL10 levels in additional JCPyV, HCMV DNAemia, and rejection

Then, we compared CXCL10 in blood at BKPyV DNAemia (>1000 copies/ml) with the CXCL10 concentration in 13 KTRs who additionally displayed JCPyV DNAemia (>1000 copies/ml), as well as in 16 KTRs with concomitant HCMV DNAemia. Of note, CXCL10 in blood showed a trend for higher concentrations in KTRs with additional JCPyV DNAemia than in patients with mere BKPyV DNAemia (median concentration: 466.03 vs. 404.44 pg/ml, 95% CI: 430.33–613.59 vs. 385.60–473.74, $P = 0.0631$, Fig. 2a). The highest CXCL10 blood concentrations were however encountered in KTRs who, in addition to BKPyV, displayed HCMV DNAemia (median concentration: 892.87 pg/ml, 95% CI: 590.29–2126.10, $P < 0.0001$ vs. BKPyV DNAemia, $P = 0.0325$ vs BKPyV and JCPyV DNAemia, $d_{cohen} = 1.50$, Fig. 2a, Table S2).

Of note, there was a significant increase of the CXCL-10 blood concentration when 10 KTRs of our study cohort displayed BKPyV DNAemia and subsequently developed additional allograft rejection (median increase: 151.94 pg/ml, 95% CI: 45.47–542.50, $P = 0.002$, Fig. 2a).

Analogously to blood levels, the CXCL-10 urine concentration was slightly higher in KTRs, who in addition to BKPyV DNAemia displayed JCPyV DNAemia than in KTRs with BKPyV DNAemia alone (median concentration: 422.15 vs. 381.15 pg/ml, 95% CI: 325.48–726.24 vs. 281.15–386.61, $P = 0.0497$, $d_{cohen} = 0.77$). In contrast to blood levels, CXCL10 in urine did not significantly differ between KTRs with BKPyV DNAemia and those who additionally displayed HCMV DNAemia (median concentration: 384.59 pg/ml, 95% CI: 259.27–485.82, $P = 0.4290$ vs. mere BKPyV, $P = 0.3291$ vs BKPyV and JCPyV DNAemia, Fig. 2b). Therefore, a significantly higher CXCL-10 blood/urine ratio was observed in KTRs with additional HCMV DNAemia, as compared to patients with BKPyV ± JCPyV DNAemia (median ratios: 1.12 for mere BKPyV, 1.10 for BKPyV and
JCPyV and 3.00 for additional HCMV DNAemia, $P = 0.008$, $d_{Cohen} = 0.90$).

In contrast and analogously to blood levels, there was a significant increase of the CXCL-10 urine concentration when 10 KTRs with BKPyV DNAemia subsequently developed additional allograft rejection (median increase: 81.22 pg/ml, 95% CI: 39.78–169.11, $P = 0.0020$, Fig. 2b).

**CXCL10 differences in relation to BKPyV-associated clinical manifestations**

Next, we analyzed whether CXCL10 in blood and urine differed among KTRs with respect to the most severe clinical manifestation which concomitantly emerged with BKPyV DNAemia during the follow-up. Therefore, KTRs with episodes of BKPyV DNAemia were grouped as shown in Table 2. In 20 KTRs, the most severe manifestation of BKPyV (+JCPyV) replication was histological evidence of PVAN; in 12 KTRs, in whom no biopsy was performed, the most severe clinical manifestation was a decrease of eGFR. In these patients, the simultaneously detected decoy cell levels were high ($>30\%$), while in 9 KTRs, in contrast, BKPyV DNAemia was associated with no significant levels of decoy cells ($<5\%$) and eGFR remained stable. Although BKPyV DNA loads exceeded 1000 copies/ml in these patients, viral loads were significantly lower than in KTRs with histological and/or clinical evidence of PVAN (Table 2). Absolute eGFR values at the time point of BKPyV DNAemia were also significantly lower in the 20 KTRs with histologically verified PVAN than in the nine patients with low decoy cell levels and lower BKPyV DNA loads (median difference: $-11.69\text{ ml/min/1.73 m}^2$, 95% CI: $-23.88$ to $-1.92$, $P = 0.0213$, $d_{Cohen} = 0.96$).

As shown in Fig. 3a and Table S2, CXCL10 in blood was significantly lower in KTRs with lower DNA loads, lower decoy cell levels ($<5\%$), and no eGFR decrease, as compared to KTRs with higher viral loads, higher decoy cell levels and a simultaneous decrease of eGFR (median concentration: 266.97 vs. 436.02 pg/ml, 95% CI: 196.09–362.66 vs. 356.77–547.10, $P = 0.0422$). Peak CXCL-10 blood concentrations, however, were observed...
in KTRs in whom PVAN was histologically verified (median concentration: 426.42 pg/ml, 95% CI: 375.39–557.33, \( P = 0.0282, d_{\text{Cohen}} = 0.86 \)).

In addition, CXCL10 in urine was higher during BKPyV DNAemia in KTRs who displayed higher DNA loads, significant levels of decoy cells and an eGFR decrease than in KTRs who did not (median concentration: 345.42 vs. 168.09 pg/ml, 95% CIs: 254.73–538.69 vs. 115.93–234.00, \( P = 0.0447 \)) and peaked in patients in whom PVAN was verified histologically (median concentration: 412.97 pg/ml, 95% CI: 291.90–571.03, \( P = 0.0204, d_{\text{Cohen}} = 0.885, \) Fig. 3b).

**Table 2.** Patient groups based on the clinical severity of BKPyV-associated manifestation during the follow-up.

| Groups and differentiation criteria | Decoy cells < 5%, no eGFRdrop | eGFR drop | Histologically verified PVAN | Difference among the groups (\( P \)) |
|------------------------------------|------------------------------|-----------|-----------------------------|----------------------------------|
| Number of patients (\( n \))       | 9                            | 12        | 20                          | <0.0001                          |
| BKPyV DNA in blood (median, range; copies/ml) | 2.0 \( \times 10^3 \), 1.0 \( \times 10^3-2.7 \times 10^4 \) | 3.2 \( \times 10^4 \), 5.3 \( \times 10^3-1.1 \times 10^6 \) | 1.4 \( \times 10^5 \), 1.1 \( \times 10^5-4.2 \times 10^8 \) |                  |
| Decoy cells in urinary sediment (median, range; %) | 1, 0-5 | 45, 30-95 | 90, 30-98                   | <0.0001                          |
| Drop of renal function (\( \geq 15\% \) decrease of eGFR, as compared to mean of the three preceding measurements) | 0/9 | 12/12 | 17/20 | <0.0001                          |
| PVAN verified in histology (biopsy within 1 week before/after sample acquisition) | No biopsy performed | No biopsy performed | 20/20 | <0.0001                          |

BKPyV, BK polyomavirus; eGFR, estimated glomerular filtration rate; PVAN, polyomavirus-associated nephropathy.
Association of CXCL10 with high-level BKPyV DNAemia and BKPyV-associated disease

Then, we analyzed CXCL10 levels in high-level BKPyV DNAemia (presumptive for PVAN) using 10 000 copies/ml (but not decoy cell levels or an eGFR decrease) as reference. Out of the 56 KTRs with peak BKPyV DNA levels >1000 copies/ml, 42 KTRs displayed viral loads >10 000 copies/ml, while 14 patients did not. CXCL10 serum concentrations were significantly higher in these 42 KTRs with peak DNA loads >10 000 copies/ml than in the 14 KTRs with lower viral loads (median CXCL10 concentration: 411.22 vs. 310.15 pg/ml, 95% CI: 438.77–162.82 vs. 318.64–116.81, P = 0.0122, $d_{Cohen} = 0.70$). For CXCL10 in urine, however, no statistically significant difference was observed (median concentration: 343.50 vs. 292.91 pg/ml, 95% CI: 258.75–366.52 vs. 194.23–414.98, P = 0.7152). As shown in Fig. S1, we also performed ROC analyses to evaluate the association of the CXCL-10 concentration with manifestations of BKPyV-associated disease. Of note, the area under the curve (AUC) was slightly higher when clinical manifestations and/or histological evidence PVAN rather than high-level BKPyV DNAemia >10 000 copies/ml were used as a reference (Table 2; Fig. S1).
CCL8, CXCL16, and CCL20 blood levels in relation to BKPyV-associated clinical manifestations

Next, we investigated whether concentrations of other T-cell associated chemokines differed in relation to the severity of BKPyV replication. Therefore, we quantified CCL8, CXCL16, and CCL20 in blood in the groups of KTR as shown in Table 2 and found that CCL8 blood concentrations were significantly higher in KTRs with BKPyV DNAemia who displayed higher viral loads, and higher decoy cell levels (n = 12) as well as histologically confirmed PVAN (n = 20), as compared to KTRs who display lower BKPyV DNA loads, lower levels of decoy cells, and no concurrent eGFR decrease (n = 9). (b) CXCL16 and (c) CCL20 in blood do not significantly differ in KTRs with BKPyV DNAemia in relation to levels of BKPyV DNA, decoy cells, the concurrent presence of an eGFR decrease as well as to histological evidence of PVAN.

Discussion

In this study, we longitudinally analyzed CXCL10 in KTRs during different stages of BKPyV replication and demonstrate that, along with progression of BKPyV replication, CXCL10 in blood and urine gradually increases, from viral DNAuria over low- and high-level DNAemia to a decrease of renal function and histological evidence PVAN. These findings suggest that CXCL10 concentrations in blood and urine might reflect the step-wise increase of inflammation and development of polyomavirus-associated disease in KTRs, a fact that should specifically be considered when CXCL10 is proposed as particular marker of allograft rejection [25,31–37].

Indeed, CXCL10 attracts and activates T-cells with a Th1 cytokine profile, and since its production can again be induced upon stimulation with Th1-derived cytokines like interferon-γ (IFN-γ), it apparently participates in a positive feedback loop [24,25]. CXCL10 is thus essential to elicit a Th1-shaped cellular immune response against viruses, but on the other hand, contributes to tissue inflammation and injury [38]. Consequently, CXCL10 has been proposed as marker of immune activation, severity, and therapy response in diverse viral diseases but also of allograft rejection [21,25,30–36].

In previous studies, elevated CXCL10 urine concentrations were equally detected when KTRs displayed BKPyV nephritis or DNAemia as well as allograft rejection [33,34,39]. Elevated blood concentrations were encountered in KTRs with BKPyV detection in urine and/or serum [8,33,40]. Our present data are in
accordance with and extend these former findings, indicating that a CXCL10 increase due to BKPyV can be equally detected in urine and blood, occurs stepwise and in proportion to the extent of viral replication, and correlates with a decrease of renal function and histological verification of PVAN. Of note, a similar observation has been made for Interleukin-6, which increases with rising BKPyV loads [41]. The gradual CXCL10 response we observed here, not only discloses that intrarenal BKPyV replication and CXCL10 secretion into urine and blood are functionally linked, but suggests its cumulative inflammatory effect on the occurrence of nephropathy. A correlation of urinary CXCL10 levels with tubule-interstitial inflammation and grade of tubulitis was previously reported, while upregulation of CXCL10 genes could pose the underlying functional background in patients with PVAN [33,42–44].

Our particular finding that not only urine but also blood CXCL10 concentrations were elevated over baseline during mere BKPyV DNAuria, furthermore highlights that even early, clinically silent, and locally restricted BKPyV replication in the allograft is sufficient to elicit a systemic CXCL10 rise and trigger inflammation. Since increased CXCL10 levels have been associated with long-term allograft dysfunction in KTRs, an increased CXCL10 concentration due to low level but sustained BKPyV replication could also possibly adversely influence allograft survival [33,35,36,39,45,46]. Correspondingly, a constantly low CXCL10 urinary concentration was recently correlated with histologically stable allografts and freedom of rejection [47]. Thus, it is indeed possible that CXCL10 might serve as inflammatory marker to individualize immunosuppressive treatment, this, however, requires further studies.

Although we demonstrated that CXCL10 gradually increases along with BKPyV replication, the potential use of CXCL10 as biomarker for PVAN may be limited. Of note, allograft rejection may also be associated with increased CXCL10 levels, whereby multiple studies have proposed CXCL10 as a rejection marker [25,31,32,39,42,46]. In this regard, our observation that CXCL10 in blood and urine already increased for mere BKPyV DNAuria, particularly suggests that absence of BKPyV should be verified, before a CXCL10 rise can be considered an indicator of allograft rejection. Indeed, we observed that CXCL10 further increased when KTRs with BKPyV DNAemia subsequently developed allograft rejection, but considering that CXCL10 responses might vary among individuals, it seems questionable whether quantitative differences might ultimately discriminate between PVAN and rejection.

With this regard, we also demonstrated that other viral agents influence the CXCL10 concentration in KTRs, and especially HCMV DNAemia showed a strong cumulative effect on CXCL10 blood but not urine levels, an observation that is in line with recent findings that CXCL10 in blood but not in urine increased in KTRs with histologically verified HCMV infection [33]. Of note, we previously demonstrated that intrapulmonary HCMV replication in lung transplant recipients caused a systemic rise of CXCL10 in blood, while in bronchoalveolar fluid it only increased when inflammatory airway obstruction simultaneously occurred [21]. In the KTRs with HCMV DNAemia we investigated, the renal allograft might thus not have been the primary site of replication or inflammation.

Finally, it is noteworthy that among the T-cell-associated chemokines, we investigated, only CXCL10 and CCL8 significantly correlated with progression of BKPyV replication, while CCL20 and CXCL16 did not. Elevated CXCL16 concentrations were found in KTRs with hypertensive kidney injury, while increased CCL20 levels were detected in clinically manifest allograft rejection [48,49]. Interestingly, in vitro data demonstrated that renal tubular epithelial cells, when stimulated with IFN-γ, strongly upregulate CXCL10 but not CCL20 [50]. Thus, due to the effect of virally induced Th1 cytokines, CXCL10 might predominate over CCL20 during BKPyV replication, indicating that different virus infections might indeed elicit different patterns of chemokine responses.

The lack of baseline samples and histological data for the whole cohort certainly pose limitations of this study. Nonetheless, in summary, the provided data demonstrate that CXCL10 in urine but not in blood already rises during early BKPyV replication, gradually increases in proportion with the extent of viral replication and correlates with the progression toward PVAN.

**Authorship**

LW: designed the study, wrote the paper, performed the research and analyzed data. LH, YW and SWA: performed the research. RS and IH: participated in research design. GB and KH: participated in research design and wrote the paper.

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Conflicts of interest

The authors declare no conflicts of interest.

REFERENCES

1. Chesters PM, Heritage J, McCance DJ. Persistence of DNA sequences of BK virus and JC virus in normal human tissues and in diseased tissues. J Inf Dis 1983; 147: 676.
2. Hirsch HH, Randhawa P. BK poliovirus in solid organ transplantation. Am J Transplant 2013; 13(Suppl 4): 179.
3. Hirsch HH, Babel N, Comoli P, et al. European perspective on human polyomavirus infection, replication and disease in solid organ transplantation. Clin Microbiol Infect 2014; 20(Suppl 7): 74.
4. Sawinski D, Goral S. BK virus infection: an update on diagnosis and treatment. Nephrol Dial Transplant 2015; 30: 209.
5. Delbue S, Ferrareso M, Ghio L, et al. A review on JC virus infection in kidney transplant recipients. Clin Dev Immunol 2013; 2013: 926391.
6. Schachtner T, Muller K, Stein M, et al. BK virus-specific immunity kinetics: a predictor of recovery from polyomavirus BK-associated nephropathy. Am J Transplant 2011; 11: 2443.
7. Schachtner T, Stein M, Babel N, Reinke P. The loss of BKV-specific immunity from pretransplantation to posttransplantation identifies kidney transplant recipients at increased risk of BKV replication. Am J Transplant 2015; 15: 2159.
8. Schachtner T, Stein M, Sefrin A, Babel N, Reinke P. Inflammatory activation and recovering BKV-specific immunity correlate with self-limited BKV replication after renal transplantation. Transpl Int 2014; 27: 290.
9. Hirsch HH, Knouse W, Dickenmann M, et al. Prospective study of polyomavirus type BK replication and nephropathy in renal-transplant recipients. New Engl J Med 2002; 347: 488.
10. Bressollette-Bodin C, Coste-Burel M, Hourmant M, Sebille V, Andre-Garnier E, Imbert-Marcille BM. A prospective longitudinal study of BK virus infection in 104 renal transplant recipients. Am J Transplant 2005; 5: 1926.
11. Pang XL, Doucette K, LeBlanc B, Cockfield SM, Preiksaitis JK. Monitoring of polyomavirus BK virus viruria and viremia in renal allograft recipients by use of a quantitative real-time PCR assay: one-year prospective study. J Clin Microbiol 2007; 45: 3568.
12. Helanterta I, Ortiz F, Auvinen E, et al. Polyomavirus BK and JC infections in well matched Finnish kidney transplant recipients. Transpl Int 2009; 22: 688.
13. Drachenberg CB, Hirsch HH, Papadimitriou JC, et al. Polyomavirus BK versus JC replication and nephropathy in renal transplant recipients: a prospective evaluation. Transplantation 2007: 84: 323.
14. Viscount HB, Eid AJ, Espy MJ, et al. Polyomavirus polymerase chain reaction as a surrogate marker of polyomavirus-associated nephropathy. Transplantation 2007; 84: 340.
15. Nickeleit V, Klimkait T, Binet IF, et al. Testing for polyomavirus type BK DNA in plasma to identify renal-allograft recipients with viral nephropathy. New Engl J Med 2000; 342: 1309.
16. Nickeleit V, Singh HK. Polyomaviruses and disease: is there more to know than viremia and viruria? Curr Opin Organ Transplant 2015; 20: 348.
17. Dadhania D, Snopkowski C, Ding R, et al. Epidemiology of BK virus in renal allograft recipients: independent risk factors for BK virus replication. Transplantation 2008; 86: 521.
18. Dadhania D, Snopkowski C, Ding R, et al. Validation of noninvasive diagnosis of BK virus nephropathy and identification of prognostic biomarkers. Transplantation 2010; 90: 189.
19. Sallusto F, Baggiolini M. Chemokines and leukocyte traffic. Nat Immunol 2008; 9: 949.
20. Babel N, Volk HD, Reinke P. BK polyomavirus infection and nephropathy: the virus-immune system interplay. Nat Rev Nephrol 2011; 7: 399.
21. Weseslindtner L, Nachbagauer R, Kundi M, et al. Human cytomegalovirus infection in lung transplant recipients triggers a CXCL-10 response. Am J Transplant 2011; 11: 542.
22. Weseslindtner L, Gorzer I, Kung E, et al. High CXCL-16 levels correlate with symptomatic disease in lung transplant recipients with human cytomegalovirus replication in the allograft. Am J Transplant 2014; 14: 2406.
23. Lisboa LF, Egli A, Fairbanks J, et al. CCL8 and the immune control of cytomegalovirus in organ transplant recipients. Am J Transplant 2015; 15: 1882.
24. Loetscher M, Gerber B, Loetscher P, et al. Chemokine receptor specific for IP10 and mig: structure, function, and expression in activated T-lymphocytes. J Exp Med 1996; 184: 963.
25. Romagnani P, Crescioli C. CXCL10: a candidate biomarker in transplantation. Clin Chim Acta 2012; 413: 1364.
26. Dufour JH, Dziezman M, Liu MT, Leung JH, Lane TE, Luster AD. IFN-gamma-inducible protein 10 (IP-10; CXCL10)-deficient mice reveal a role for IP-10 in effector T cell generation and trafficking. J Immunol 2002; 168: 3195.
27. Weseslindtner L, Gorzer I, Roedl K, et al. Intrapulmonary human cytomegalovirus replication in lung transplant recipients is associated with a rise of CCL-18 and CCL-20 chemokine levels. Transplantation 2017; 101: 197.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Association of CXCL10 with high level BKPyV DNAemia and BKPyV associated disease.
Table S1. Clinical information of study patients.
Table S2. Quantitative values of CXCL10 measurements.
Appendix S1. Material and Methods.
28. Masutani K, Shapiro R, Basu A, Tan H, Wijkstrom M, Randhawa P. The Banff 2009 Working Proposal for polyomavirus nephropathy: a critical evaluation of its utility as a determinant of clinical outcome. Am J Transplant 2012; 12: 907.

29. Loupy A, Haas M, Solez K, et al. Banff 2015 kidney meeting report: current challenges in rejection classification and prospects for adopting molecular pathology. Am J Transplant 2017; 17: 28.

30. Reiberger T, Aberle JH, Kundi M, et al. IP-10 correlates with hepatitis C viral load, hepatic inflammation and fibrosis and predicts hepatitis C virus relapse or non-response in HIV-HCV coinfection. Antivir Ther 2008; 13: 969.

31. Blydt-Hansen TD, Gibson IW, Gao A, Dufault B, Ho J. Elevated urinary CXCL10-to-creatinine ratio is associated with subclinical and clinical rejection in pediatric renal transplantation. Transplantation 2015; 99: 797.

32. Hirt-Minkowski P, De Serres SA, Ho J. Developing renal allograft surveillance strategies - urinary biomarkers of cellular rejection. Can J Kidney Health Dis 2015; 2: 28.

33. Ho J, Schaub S, Wiebe C, et al. Urinary CXCL10 chemokine is associated with alloimmune and virus compartment-specific renal allograft inflammation. Transplantation 2018; 102: 521.

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

35. Lazzi E, Rotondi M, Mazzinghi B, et al. High CXCL10 expression in rejected kidneys and predictive role of pretransplant serum CXCL10 for acute rejection and chronic allograft nephropathy. Transplantation 2005; 79: 1215.

36. Rabant M, Amrouche L, Lebreton X, et al. Urinary C-X-C motif chemokine 10 independently improves the noninvasive diagnosis of antibody-mediated kidney allograft rejection. J Am Soc Nephrol 2015; 26: 2840.

37. Fairchild RL, Suthanthiran M. Urine CXCL10/IP-10 finger's ongoing antibody-mediated kidney graft rejection. J Am Soc Nephrol 2015; 26: 2607.

38. Qin S, Rottman JB, Myers P, et al. The chemokine receptors CXCR3 and CCR5 mark subsets of T cells associated with certain inflammatory reactions. J Clin Invest 1998; 101: 746.

39. Hu H, Aizenstein BD, Puchalski A, Burmania JA, Hamawy MM, Knechtle SJ. Elevation of CXCR3-binding chemokines in urine indicates acute renal-allograft dysfunction. Am J Transplant 2004; 4: 432.

40. Kariminik A, Dabiri S, Yaghobi R. Polyomavirus BK induces inflammation via up-regulation of CXCL10 at translation levels in renal transplant patients with nephropathy. Inflammation 2016; 39: 1514.

41. Sadeghi M, Daniel V, Schnitzler P, et al. Urinary proinflammatory cytokine response in renal transplant recipients with polyomavirus BK viruria. Transplantation 2009; 88: 1109.

42. Hirt-Minkowski P, Amico P, Ho J, et al. Detection of clinical and subclinical tubulo-interstitial inflammation by the urinary CXCL10 chemokine in a real-life setting. Am J Transplant 2012; 12: 1811.

43. 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.

44. Tatapudi RR, Muthukumar T, Dadhana D, et al. Noninvasive detection of renal allograft inflammation by measurements of mRNA for IP-10 and CXCR3 in urine. Kidney Int 2004; 65: 2390.

45. Hirt-Minkowski P, Ho J, Gao A, et al. Prediction of long-term renal allograft outcome by early urinary CXCL10 chemokine levels. Transplant Direct 2015; 1: e31.

46. 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.

47. Rabant M, Amrouche L, Morin L, et al. Low urinary CXCL9 and CXCL10 might predict immunological quiescence in clinically and histologically stable kidney recipients. Am J Transplant 2016; 16: 1868.

48. Woltman AM, de Fijter JW, van der Kooij SW, et al. MIP-3alpha/CCL20 in renal transplantation and its possible involvement as dendritic cell chemoattractant in allograft rejection. Am J Transplant 2005; 5: 2114.

49. Xia Y, Entman ML, Wang Y. Critical role of CXCL16 in hypertensive kidney injury and fibrosis. Hypertension 2013; 62: 1129.

50. Demmers MW, Baan CC, van Beelen E, Ijzermans JN, Weimar W, Rowshani AT. Differential effects of activated human renal epithelial cells on T-cell migration. PLoS ONE 2013; 8: e64916.