

Supplemental Appendix for

**Deciphering the prognostic and predictive value of urinary CXCL10 in kidney recipients
with BK virus reactivation**

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SUPPLEMENTAL RESULTS

Supplemental Table 1: Oligo sequences and probes used for transcript quantification by qPCR.

Gene	Reference	Sequence	Localization
BKV VP1	J02038	Sense: 5'-TGCTGATATTTGTGGCCTGTTTACTA-3'	2355-2380
	K00058	Antisense: 5'-CTCAGGCGGATCTTAAAATATCTTG-3'	2438-2414
	V01108	Probe: 5-FAM-AGCTCTGGAACACAACAGTGGAGAGGCC TAMRA 3'	2383-2410
BAK	Y13231	Sense: 5' CCCACATCTGGAGCAGAGTCA 3'	192-212
		Antisense: 5' CAGATGCCATTTTCAGGTCTTG 3'	264-242
		5' FAM CAGGTGACAAGTGACGGTGGTCTCCA TAMRA 3'	215-240

Supplemental Table 2: Patient and transplant characteristics in the cross-sectional study.

Variables	N=391 patients
Recipient characteristics	
Men, <i>n (%)</i>	241 (61.6)
Age at transplantation (yr), <i>mean±SD</i>	47.6±15.7
Cause of ESRD	
GN, <i>n (%)</i>	88 (22.5)
Diabetes, <i>n (%)</i>	40 (10.2)
Cystic/hereditary/congenital, <i>n (%)</i>	91 (23.3)
Secondary GN, <i>n (%)</i>	21 (5.4)
Hypertension, <i>n (%)</i>	25 (6.4)
Interstitial nephritis, <i>n (%)</i>	43 (11.0)
Miscellaneous conditions, <i>n (%)</i>	6 (1.5)
Cancer, <i>n (%)</i>	0
Etiology uncertain, <i>n (%)</i>	77 (19.7)
Transplant variables	
Donor age (yr), <i>mean±SD</i> ^a	53.8±17.2
Donor type ^b	
Living donor, <i>n (%)</i>	107 (27.9)
SCD, <i>n (%)</i>	129 (33.6)
ECD, <i>n (%)</i>	148 (38.5)
Retransplantation, <i>n (%)</i>	83 (21.2)
Delayed graft function, <i>n (%)</i>	169 (43.7)
Preformed DSAs, <i>n (%)</i> ^c	178 (46.5)
Immunosuppressive protocol	
Induction therapy, <i>n (%)</i> ^d	369 (98.3)
Basiliximab/Thymoglobuline®, <i>n (%)</i>	186 (49.9)/178 (47.7)
Calcineurin inhibitor, <i>n (%)</i> ^e	379 (98.2)
Cyclosporin/tacrolimus, <i>n (%)</i>	72 (18.7)/307 (79.5)
Purine synthesis inhibitor, <i>n (%)</i> ^e	363 (94.0)
Azathioprine/mycophenolic acid, <i>n (%)</i>	10 (2.6)/353 (91.5)
Mammalian target of rapamycin inhibitor, <i>n (%)</i> ^e	28 (7.3)
Steroids, <i>n (%)</i> ^e	383 (99.2)

Abbreviations: DSAs, donor-specific antibodies; ECD, expanded-criteria donor; ESRD, end-stage renal disease; GN, glomerulonephritis; SCD, standard-criteria donor; SD, standard deviation. ^a Data not available (NA) for 3 patients. ^b NA for 7 patients. ^c NA for 8 patients. ^d NA for 18 patients. ^e NA for 5 patients.

Supplemental Table 3: Patient and transplant characteristics in the nested case-control study including 63 single patients with BKV-DNAemia.

Variables	N=63 patients
Recipient characteristics	
Men, <i>n (%)</i>	35 (55.6)
Age at transplantation (yr), <i>mean±SD</i>	50±17.0
Cause of ESRD	
GN, <i>n (%)</i>	15 (23.8)
Diabetes, <i>n (%)</i>	7 (11.1)
Cystic/hereditary/congenital, <i>n (%)</i>	19 (30.2)
Secondary GN, <i>n (%)</i>	2 (3.2)
Hypertension, <i>n (%)</i>	5 (7.9)
Interstitial nephritis, <i>n (%)</i>	7 (11.1)
Miscellaneous conditions, <i>n (%)</i>	0
Cancer, <i>n (%)</i>	0
Etiology uncertain, <i>n (%)</i>	8 (12.7)
Transplant variables	
Donor age (yr), <i>mean±SD</i>	58±19.0
Donor type ^a	
Living donor, <i>n (%)</i>	16 (26.7)
SCD, <i>n (%)</i>	14 (23.3)
ECD, <i>n (%)</i>	30 (50.0)
Retransplantation, <i>n (%)</i>	10 (15.9)
Delayed graft function, <i>n (%)</i>	26 (41.3)
Preformed DSAs, <i>n (%)</i>	32 (50.8)
Immunosuppressive protocol	
Induction therapy, <i>n (%)</i> ^b	59 (100)
Basiliximab/Thymoglobuline®, <i>n (%)</i>	24 (40.7)/34 (57.6)
Calcineurin inhibitor, <i>n (%)</i>	62 (98.4)
Cyclosporin/tacrolimus, <i>n (%)</i>	5 (7.9)/57 (90.5)
Purine synthesis inhibitor, <i>n (%)</i>	62 (98.4)
Azathioprine/mycophenolic acid, <i>n (%)</i>	1 (1.6)/61 (96.8)
Mammalian target of rapamycin inhibitor, <i>n (%)</i>	2 (3.2)
Steroids, <i>n (%)</i>	63 (100)

Abbreviations: DSAs, donor-specific antibodies; ECD, expanded-criteria donor; ESRD, end-stage renal disease; GN, glomerulonephritis; SCD, standard-criteria donor; SD, standard deviation. ^a Data not available (NA) for 3 patients. ^b NA for 4 patients

Supplemental Table 4: Sample characteristics in the nested case-control study including 63 single patients with BKV-DNAemia. The low-CXCL10 group was defined as uCXCL10/cr ≤ 12.86 ng/mmol, and the high-CXCL10 group was defined as uCXCL10/cr > 12.86 ng/mmol. MVI is defined by the sum of the glomerulitis and peritubular capillaritis scores.

Variables	All patients (N=63)	Low-CXCL10 group (N=40)	High-CXCL10 group (N=23)	P value
Time from transplantation to biopsy (mo), median (IQR)	10 (19.0)	13 (25.5)	6 (8.0)	0.003
Indication of biopsy				
Screening biopsy, n (%)	4 (6.3)	2 (5.0)	2 (8.7)	0.97
Clinically indicated biopsy, n (%)	59 (93.7)	38 (95.0)	21 (91.3)	
Rise in serum creatinine, n (%)	23 (39.0)	16 (42.1)	7 (33.3)	0.70
Proteinuria, n (%)	0	0	0	
De novo DSAs, n (%)	0	0	0	
Control after rejection, n (%)	1 (1.7)	0	1 (4.8)	0.76
BKV-DNAemia, n (%)	35 (59.3)	22 (57.9)	13 (61.9)	0.98
Other, n (%)	0	0	0	
Banff elementary lesions				
i score, mean \pm SD ^a	0.21 \pm 0.60	0.15 \pm 0.54	0.30 \pm 0.70	0.14
t score, mean \pm SD	0.90 \pm 1.29	0.78 \pm 1.21	1.13 \pm 1.42	0.37
ci score, mean \pm SD ^a	1.16 \pm 1.07	1.21 \pm 1.08	1.09 \pm 1.08	0.67
ct score, mean \pm SD ^a	1.15 \pm 1.05	1.18 \pm 1.05	1.09 \pm 1.08	0.71
i-IFTA score, mean \pm SD	0.75 \pm 1.09	0.80 \pm 1.07	0.65 \pm 1.15	0.45
ti score, mean \pm SD	0.54 \pm 0.96	0.53 \pm 0.96	0.57 \pm 0.99	0.92
g score, mean \pm SD	0.24 \pm 0.64	0.28 \pm 0.68	0.17 \pm 0.58	0.38
ptc score, mean \pm SD	0.21 \pm 0.60	0.10 \pm 0.44	0.39 \pm 0.78	0.02
MVI score, mean \pm SD	0.44 \pm 1.03	0.38 \pm 0.90	0.57 \pm 1.24	0.56
cg score, mean \pm SD	1.10 \pm 0.35	1.10 \pm 0.38	1.09 \pm 0.29	0.89
ah score, mean \pm SD	2.40 \pm 1.06	2.53 \pm 1.01	2.17 \pm 1.11	0.21
cv score, mean \pm SD ^b	2.57 \pm 1.02	2.57 \pm 1.01	2.35 \pm 1.04	0.35
Pathologic primary diagnosis				
Inadequate, n (%)	4 (6.3)	2 (5.0)	2 (8.7)	0.97
BKVN, n (%)	15 (23.8)	11 (27.5)	4 (17.4)	0.55
Acute rejection, n (%)	9 (14.3)	4 (10.0)	5 (21.7)	0.36
Normal, n (%)	3 (4.8)	2 (5.0)	1 (4.4)	0.62
Other lesions, n (%) ^c	33 (52.4)	22 (55.0)	11 (47.8)	0.77
BKV infection characteristics				
Time from 1 st blood BKV-positive PCR to biopsy (d), median (IQR)	43 (113.0)	45.5 (127.8)	43 (70.5)	0.84

Time from peak DNAemia to biopsy (d), <i>median (IQR)</i>	0 (40.5)	0 (44.3)	0 (54.0)	0.20
BKV viral load at biopsy (Log_{10} copies/mL), <i>median (IQR)</i>	3.4 (1.8)	3.2 (1.9)	3.4 (1.7)	0.59
Peak BKV viral load (Log_{10} copies/mL), <i>median (IQR)</i>	4.6 (1.8)	4.4 (1.8)	4.8 (1.5)	0.16
Laboratory test results at the time of biopsy				
Serum creatinine ($\mu\text{mol/L}$), <i>mean\pmSD</i>	163 \pm 51	162 \pm 53	165 \pm 47	0.67
DSAs, <i>n (%)</i> ^d	22 (34.9)	11 (30.6)	11 (47.8)	0.29
Proteinuria/creatininuria ratio (g/g), <i>mean\pmSD</i>	0.38 (0.6)	0.37 (0.6)	0.39 (0.4)	0.19
Urinary CXCL10 (LnCXCL10/cr), <i>median (IQR)</i>	2.21 (1.2)	1.94 (1.3)	3.02 (0.8)	<0.0001

Abbreviations: ah, arteriolar hyalinosis; BKVN, BKV-associated nephropathy; cg, allograft glomerulopathy; ci, interstitial fibrosis; ct, tubular atrophy; cv, chronic vascular changes; DSAs, donor-specific antibodies; g, glomerulitis; i, interstitial infiltrate; IFTA, interstitial fibrosis/tubular atrophy; IQR, interquartile range; Ln, natural logarithm; MVI, microvascular inflammation; PCR, polymerase chain reaction; ptc, peritubular capillaritis; SD, standard deviation; t, tubulitis; ti, total inflammation; v, vasculitis. ^a Data not available (NA) for 1 case, ^b NA=8, ^c Including calcineurin inhibitor toxicity, IFTA and recurrent disease, ^d NA=4.

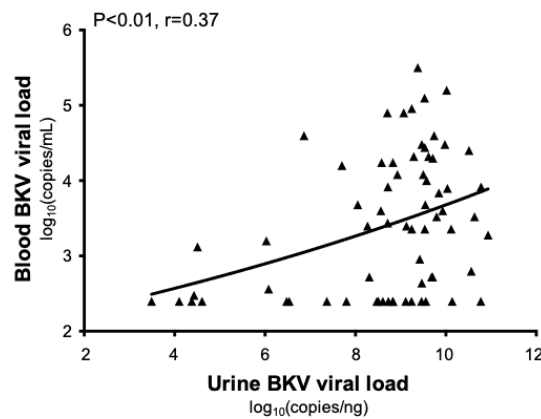
Supplemental Table 5: Patient and transplant characteristics in the longitudinal study including 60 single patients with BKV-DNAemia.

Variables	N=60 patients
Recipient characteristics	
Men, <i>n (%)</i>	36 (60.0)
Age at transplantation (yr), <i>mean±SD</i>	53.6 (15)
Cause of ESRD	
GN, <i>n (%)</i>	15 (25.0)
Diabetes, <i>n (%)</i>	5 (8.3)
Cystic/hereditary/congenital, <i>n (%)</i>	24 (40.0)
Secondary GN, <i>n (%)</i>	4 (6.7)
Hypertension, <i>n (%)</i>	1 (1.7)
Interstitial nephritis, <i>n (%)</i>	2 (3.3)
Miscellaneous conditions, <i>n (%)</i>	2 (3.3)
Etiology uncertain, <i>n (%)</i>	7 (11.7)
Transplant variables	
Donor age (yr), <i>mean±SD</i>	56.3 (18)
Donor type	
Living donor, <i>n (%)</i>	13 (21.7)
SCD, <i>n (%)</i>	16 (26.7)
ECD, <i>n (%)</i>	29 (48.3)
DCD, <i>n (%)</i>	2 (3.3)
Retransplantation, <i>n (%)</i>	17 (28.3)
Delayed graft function, <i>n (%)</i>	8 (13.3)
Preformed DSAs, <i>n (%)</i>	39 (65.0)
Immunosuppressive protocol	
Induction therapy, <i>n (%)</i>	
Basiliximab/Thymoglobuline®, <i>n (%)</i>	22 (36.7)/38 (63.3)
Calcineurin inhibitor, <i>n (%)</i>	
Cyclosporin/tacrolimus, <i>n (%)</i>	0 (0)/60 (100)
Mycophenolic acid, <i>n (%)</i>	55 (91.7)
Mammalian target of rapamycin inhibitor, <i>n (%)</i>	5 (8.3)
Steroids, <i>n (%)</i>	60 (100)

Abbreviations: DCD, donation after cardiac death; DSAs, donor-specific antibodies; ECD, expanded-criteria donor; ESRD, end-stage renal disease; GN, glomerulonephritis; SCD, standard-criteria donor; SD, standard deviation.

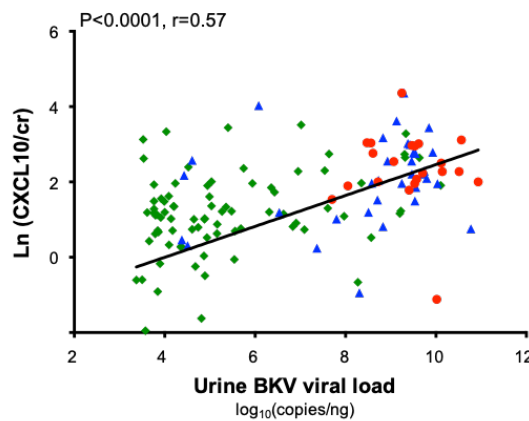
Supplemental Figure 1: Correlation analyses in the cross-sectional study. **(A)** Correlation between blood and urine BKV viral load. **(B)** Correlation between uCXCL10 and urine BKV viral load in a restricted population after exclusion of possible confounders. **(C)** Correlation between uCXCL10 and urine protein output in all samples with BKV DNAemia. Correlations were computed using Pearson's tests. Abbreviations: UTI, urinary tract infection.

A



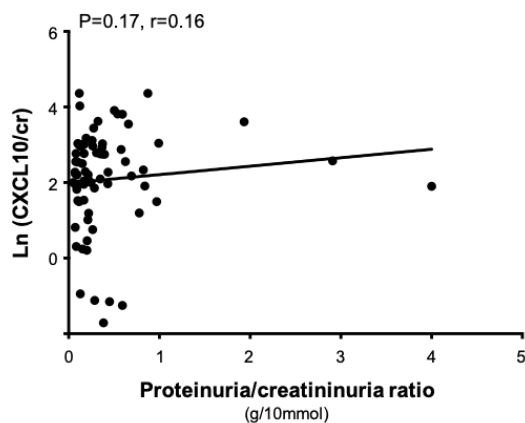
Global population of patients with viremia: N=76 samples of whom 68 have concurrent viruria, see Fig 2A)

B



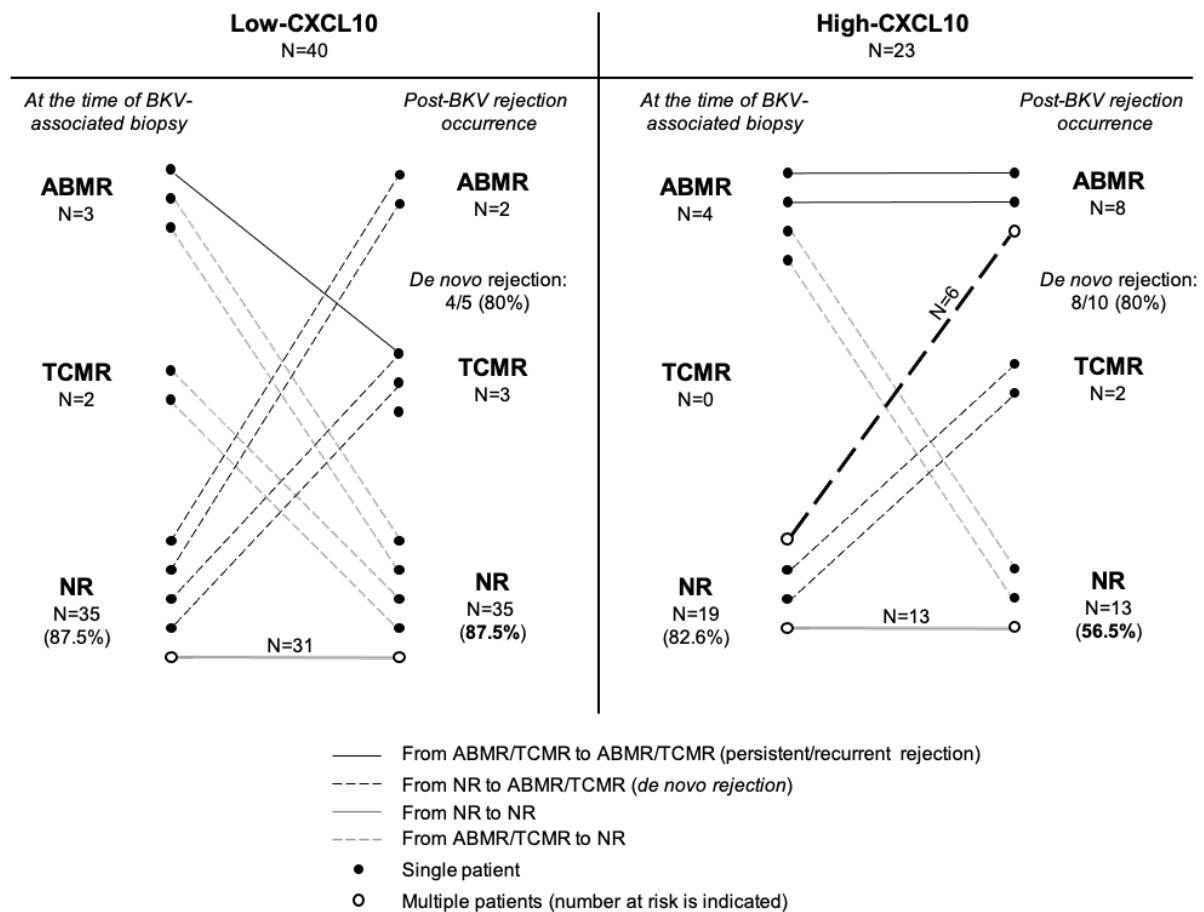
Restricted population of patients with viremia: N=158 samples (after exclusion of N=13 UTI cases and/or N=35 acute rejection cases)

C



Global population of patients with viremia: N=76 samples of whom proteinuria was available for 74 samples

Supplemental Figure 2: Relationship between the rejection phenotypes observed at the time of index biopsy for concurrent BKV-DNAemia and the post-BKV occurrence of rejection in the low-CXCL10 (≤ 12.86 ng/mmol) and high-CXCL10 (>12.86 ng/mmol) groups from the nested case-control study. Dashed lines indicate a change in category from no rejection to rejection (i.e., *de novo* rejection, in black) or the opposite from rejection to no rejection (in gray). Solid lines indicate patients staying within the same category: with a rejection diagnosis at the time of index biopsy and a post-BKV persistent/recurrent rejection (in black) or no rejection at any time (in gray).



Supplemental Figure 3: Urinary CXCL10/cr (A) and blood BKV viral load (B) trajectory analyses in the longitudinal cohort split in three groups according to AST Guidelines (Hirsch *et al*, Clin Transplant 2019). Transient BKV-DNAemia is defined by spontaneous BKV clearance without lowering of maintenance immunosuppression (N=18, light gray line). Sustained low-level BKV-DNAemia is defined by peak viremia <4 log₁₀c/mL (N=27 KTRs, dark gray line). Sustained high-level DNAemia is defined by peak viremia ≥4 log₁₀c/mL (N=13 KTRs, black line). Trajectories were computed by regression from longitudinal assessments of uCXCL10/cr (samples collected at biopsy and each outpatient clinic visit during the 1st year post-transplantation) and all available blood BKV viral loads over the same period.

