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The impact of surveillance and rapid reduction in immunosuppression to control BK virus-related graft injury in kidney transplantation

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Summary

We prospectively screened 609 consecutive kidney (538) and kidney-pancreas (71) transplant recipients for BK viremia over a 4-year interval using polymerase chain reaction viral load detection and protocol kidney biopsies. We found that BK viremia is common at our center: total cases 26.7%, cases during first year 21.3% (mean 4 months), and recipients with ≥10 000 copies/ml 12.3%. We found few predictive clinical or demographic risk factors for any BK viremia or viral loads ≥10,000 copies/ml, other than prior treatment of biopsy confirmed acute rejection and/or higher immunosuppressive blood levels of tacrolimus ($P = 0.001$) or mycophenolate mofetil ($P = 0.007$). Viral loads at diagnosis (<10 000 copies/ml) demonstrated little impact on graft function or survival. However, rising copy numbers demand early reductions in immunosuppressive drug doses of at least 30–50%. Viral loads >185 000 copies/ml at diagnosis were predictive of BK virus-associated nephropathy (BKVAN; OR: 113.25, 95% CI: 17.22–744.6, $P < 0.001$). Surveillance for BK viremia and rapid reduction of immunosuppression limited the incidence of BKVAN to 1.3%. The addition of leflunomide or ciprofloxacin to immunosuppressive dose reduction did not result in greater rates of viral clearance. These data support the role of early surveillance for BK viremia to limit the impact on transplant outcome, although the most effective schedule for screening awaits further investigation.

Introduction

During the last 10 years, the BK virus (BKV) has emerged as a common post-transplant infection among kidney recipients, with detection rates from 20% to 60% in the urine or blood [1–4]. The progression to the specific BKV-associated nephropathy (BKVAN) is reported in up to 10% of cases and is a leading cause of graft dysfunction, permanent graft injury, and even graft loss [5,6]. The majority of cases have been detected during the evaluation of graft dysfunction with sampling of blood for viral particles, or urine for decoy cells [7–9]. Distinct patterns of the viral infection have been described in kidney allograft histology, which often correlate with the severity of accompanying viremia and subsequent permanent graft damage [10,11]. While some agents have been reported to have anti-viral activity, the most effective treatment of BK viremia and BKVAN defined by evidence-based trial data is lacking [6,12–14]. The best current practice is focused on the infection as representative of over immunosuppression with the need to reduce immunosuppression as the prudent first step [1,15,16].

As the pattern of BKV infection appears to evolve from lower levels of viremia to higher viral loads to graft invasion to renal dysfunction to permanent graft damage, it may be possible to avoid these later stages by screening the at-risk population and intervening at earlier stages [17–20]. In this study, we report the role of prospective screening for BK viremia in all kidney and kidney-pancreas transplant recipients at our center over the past 4–5 years. Patients were managed with several treatment options once the virus was detected independent of renal function or other graft characteristics. The impact of progressive BK viral loads at initial diagnosis was assessed with respect to eventual viral clearance, renal function, and transplant outcomes.

Materials and methods

Patients

Between January 1, 2007 and June 30, 2011, we prospectively monitored 622 kidney-only and kidney-pancreas transplant recipients for detection of the BKV using real-time polymerase chain reaction (PCR) viral load assays. This screening study was Institutional Review Board approved and included 7453 tests (11.9 per patient). All patients were free of active BKV at the time of transplant surgery. There were 609 patients who had a functioning graft for at least 30 days and completed follow-up (10 died or had graft loss <2 months; 3 were not screened), which constitutes the study population. Screening for BKV was performed monthly in the first 6 months after transplantation, then bimonthly for months 6–12. Compliance with the screening protocol was defined as ≥6 PCR values in the first year; therefore, 537/609 = 88.1% of patients were compliant with screening. Data were collected on patient demographics and clinical characteristics at the time of transplant as well as transplant outcomes.

Immunosuppression

For this study population, 68% (417) received a nondepleting antibody for induction (basiliximab), and a depleting antibody was given to 32% (192); thymoglobulin 190, alemtuzumab 1, or OKT3 1. Maintenance immunosuppression included tacrolimus (TAC)–mycophenolate mofetil (MMF)–prednisone 85% (520); sirolimus–MMF–prednisone 4.2% (26); cyclosporine–MMF–prednisone 1% (3); and steroid avoidance 9.8% (60). Target immunosuppressive drug levels for the first 6 months were, TAC 6–12 ng/ml; sirolimus 8–12 ng/ml; and MMF 2–4 mg/l. The mean blood levels of TAC and MMF were calculated from transplant until the date of diagnosis of BK viremia for the BKV PCR-positive group. The mean of all TAC and MMF blood levels was calculated from transplant until month 12 for the BKV-negative group.

Quantitative PCR for BKV-DNA detection

Serum BK viral loads were measured by Mayo Medical Laboratories, Rochester, MN. A real-time quantitative PCR assay was used targeting a region of the large T-antigen gene specific for BKV. This assay does not detect JC virus or SV40 (other polyomaviruses). The lower limit of detection is 500 target copies/ml (http://www.mayomedicallaboratories.com/test-catalog/Performance/88910).

Detection of BK viremia and BKVAN

For the entire population, the incidence of BK viremia was 26.7% (163/609) and was 21.3% (130/609) during the first 12 months. The peak months for detection were 2, 3, and 4 (Fig. 1). The 163 BK viremia positive recipients were further classified according to the highest peak viral load into BKV low viremia [<10 000 copies/ml, $n = 88$, median 1500 (IQR 10th 500–90th 7000)], and BKV high viremia [≥10 000 copies/ml, $n = 75$, median 47 000 (IQR 10th 11 840–90th 646 000)]. We assessed the long-term effect of high- and low-BK viremia in terms of serum creatinine (mg/dl) and estimated glomerular filtration rate (eGFR; aMDRD, abbreviated 4 value modification of diet in renal disease) at 3, 6 and 12 months after transplant as well as rejection rates and graft survival rates. We also analyzed the risk factors for high-BK viremia.

All our study population had an implant kidney biopsy at the time of transplant. In addition, transplant renal biopsies were done either as a 'protocol biopsy' at months 3 and 12 post-transplant, or 'for cause biopsy' in the setting of renal dysfunction. *In situ* hybridization for BKV was routinely performed for those patients with known BK viremia. Cases that demonstrated positive staining for BKV by *in situ* hybridization in the setting of positive BK viremia were diagnosed as BKVAN. In this study, we identified the risk

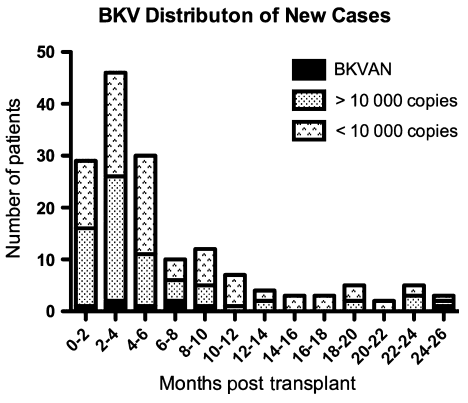

Figure 1 Distribution and copy numbers of *de novo* cases of BK viremia.

factors of BKVAN as well as the cut-off levels to predict BKVAN.

Treatment of BK viremia and BKVAN

The 130 recipients with BK viremia detected during the first year were further classified according to the treatment received into five categories. The treatments were chosen by the treating physician, and reflected disease intensity based on viral loads and clinical symptoms. These included Group 1, observation alone ($n = 45$); Group 2, reduction in immunosuppression alone ($n = 43$); Group 3, reduction in immunosuppression plus ciprofloxacin ($n = 15$); Group 4, reduction in immunosuppression + leflunomide ($n = 18$); or Group 5, reduction in immunosuppression + ciprofloxacin + leflunomide ($n = 9$). We studied the recipient characteristics in the different treatment categories, and compared the rate (slope) of BK viral load decline among the five groups. For recipients in Group 1, median BK viral load was 1000 copies/ml (IQR 10th 500–90th 4500). For recipients in Groups 2–5, the median BK viral load was 27 500 copies/ml (IQR 10th 1500–90th 599 400), and the maintenance immunosuppressive drug doses of TAC, or Cyclosporine and MMF were lowered 30–50% from the dose at first detection of BKV. In addition, Group 3 patients had ciprofloxacin 250–500 mg bid added. Group 4 patients had the MMF discontinued and replaced by Leflunomide, which was initiated at 100 mg/day for 5 days followed by a maintenance daily dose of 40 mg/day targeting teriflunomide levels at 60 000 ng/ml. Group 5 patients had their MMF discontinued and both the leflunomide and ciprofloxacin given in replacement.

Clearance of BK viremia

The BK viremia group ($n = 130$ patients) was classified into Cleared (no detection of the virus for at least 3 consecutive months: $n = 101/130$, 78%) and non-Cleared (persistent viremia for 3 consecutive months: $n = 29/130$, 22%) to identify significant factors associated with viral clearance using univariable and multivariable proportional hazard survival regression analysis.

Statistical analysis

All continuous variables were summarized as means, medians, standard deviations, and percentiles; the differences were analyzed using the two sample *T*-test, or nonparametric tests. Categorical variables were described using frequencies and percentiles, and compared using Fisher's exact test/Pearson's chi-squared test. Univariable association and univariable and multivariable proportional hazard survival regression tests were used to identify risk factors of BKV infection at any time, censored at 1 year, and for high viremia (≥10 000 copies/ml). Data were censored when a patient died, experienced graft loss, resumed dialysis or was lost to follow-up. Receiver operating characteristic (ROC) analysis using logistic regression was performed to assess the BKV copies cut-off to predict BKVAN. General linear mixed models were generated to compare the slope rates among the five different BKV treatment classes. All tests were performed at a significance level of 0.05, and SAS 9.3 software (SAS Institute, Cary, NC, USA) was used.

Results

Risk factors of BK viremia

The study included 41% (250) live donor, 47% (288) deceased donor (DD) kidney-only, and 12% (71) kidney-pancreas recipients. The demographic and clinical characteristics of the 163 recipients with BK viremia and the 446 without BK viremia appear in Table 1. The following characteristics were not associated with an increased risk to detect BK viremia at any time or censored at 1-year post-transplant using a univariate model (P = NS): recipient age, gender, race, cause end-stage renal disease (ESRD), ABO group, body mass index (BMI), transplant percent reactive antibody (PRA), smoking history, kidney-pancreas, transplant number, depleting antibody, pretransplant dialysis, CMV or EBV serology, biopsy confirmed acute rejection (BCAR), or immunosuppression (calcineurin inhibitor, mammalian target of rapamycin, steroids), donor source, age, gender, race, or ABO group. Trough blood levels of TAC (9.7 vs. 9.1 ng/ml, $P = 0.001$) and MMF (3.06 vs. 2.6 mg/l, $P = 0.007$) were found to be significantly higher prior to the initial detection BK viremia the first year, Table 2.

Risk factors for BK high-peak viremia (≥10 000 copies/ml)

The same risk factors described above were not associated with an increased risk to detect peak BK viremia ≥10 000 copies/ml at any time post-transplant using a univariate model (P = NS). We did find a significantly increased risk for BK viremia ≥10 000 copies/ml in recipients that had treated BCAR at any time 37.3% vs. 21% (BKV <10 000 copies/ml plus patients without viremia) $P = 0.002$; or the first year 18.7% vs. 10.7% (BKV <10 000 copies/ml plus patients without viremia) $P = 0.043$. To confirm this in a Cox proportional hazards regression model, we found treated BCAR at any time HR: 2.010 (95% CI: 1.096–3.685) $P = 0.020$, and treated BCAR the first year HR: 1.994 (95% CI: 1.114–3.570) $P = 0.024$ were associated with increased risk for BK viremia ≥10 000 copies/ml. In addition, pre-BKV blood levels of TAC (10.2 vs. 9.2 ng/ml, $P < 0.0001$) and MMF (3.22 vs. 2.6 mg/l, $P < 0.0054$) were found to be

Table 1. Demographic and clinical characteristics.

	BKV = positive (n = 163)		BKV = negative (n = 446)			
Factor	n	%	n	%	P-value	Total (n = 609)
Recipient age (years)						
Mean (SD)	51.6 (11.9)		49.7 (12.5)		0.1	50.25 (12.39)
Range	21–75		18–76			18–76
Recipient gender						
Male	101	62	285	64	0.66	386
Recipient race						
White	119	73	337	75	0.08	456
Black	38	23	91	20		129
Others	6	4	18	5		24
Type of donor						
Deceased	97	60	262	59	0.86	359
Living	66	40	184	41		250
Graft number						
1	144	88	385	86	0.51	529
2	19	12	61	14		80
PRA%						
>10	44	27	120	27	0.5	164
<10	119	73	326	73		445
Cause of ESRD						
DM	56	34	137	31	0.72	193
HTN	21	13	58	13		79
GN	36	22	105	24		141
APKD	23	14	55	12		78
FSGS	11	7	26	6		37
Others	16	10	65	14		81
Type of Tx						
Kidney	142	87	396	89	0.5	538
Kidney-pancreas	21	13	50	11		71
Pretransplant dialysis						
Yes	124	76	347	78	0.65	471
Recipient CMV IgG at transplant						
Positive	86	53	218	49	0.39	304
Donor gender						
Male	85	52	238	53	0.79	323
Donor age						
Mean (SD)	36.6 (14.1)		38.5 (14.1)		0.14	
Range	3–67		1–68			1–68
Biopsy confirmed acute rejection	21	13	50	11	0.5	71
Banff ≥ 1A	14	9	19	4.2		
Banff borderline	7	4	31	6.9		
Prior to BKV	9	6	NA			
Steroids						
Yes	147	90	402	90	0.98	549
No	16	10	44	10		60
Tacrolimus						
Yes	158	97	427	96	0.75	585
No	5	3	19	4		24
Sirolimus						
Yes	3	2	12	3	0.54	15
No	160	98	434	97		594
Depleting Ab for induction						
Yes	55	34	137	31	0.47	192
No	108	66	309	69		417

APKD, Adult Polycystic Kidney Disease; BKV, BK virus; CMV, Cytomegalovirus; DM, Diabetes Mellitus; FSGS, Focal Segmental Glomerulosclerosis; GN, Glomerulonephritis; HTN, Hypertension; PRA, percent reactive antibody; ESRD, end-stage renal disease; Tx, transplant.

Table 2. Tacrolimus (ng/ml) and mycophenolate mofetil (mg/l) trough blood levels among recipients with and without BK viremia; mean ± SD.

		Positive during first year post-Tx (n = 130)		
Bk viremia	Negative first year post-Tx (n = 479)	Mean drug level until the onset of BKV	Mean of last three levels before the onset of BKV	P-value
Tacrolimus (ng/ml)	9.1 ± 1.1	9.7 ± 2[a]	9.68 ± 2.55[b]	[a]0.001 [b]0.002
Mycophenolate mofetil (mg/l)	2.6 ± 1.2	2.9 ± 1.6[a]	3.06 ± 1.8[b]	[a]0.02 [b]0.007
	BKV <10 000 copies (BKV negative plus low-BK viremia) (n = 534)	High BK viremia >10 000 copies (n = 75)		
		Mean drug level until the onset of BKV	Mean of last three levels before the onset of BKV	
Tacrolimus (ng/ml)	9.2 ± 1.3	9.9 ± 1.8[a]	10.2 ± 2.4[b]	[a]<0.0001 [b]<0.0001
Mycophenolate mofetil (mg/l)	2.6 ± 1.2	2.9 ± 1.5[a]	3.22 ± 2.1[b]	[a]0.01 [b]0.0054

The P values refer to the values with the related superscript a or b.
BKV, BK virus; Tx, transplant.

significantly higher in the group that developed BK viremia ≥10 000 copies/ml, Table 2.

Clinical significance of high peak BK viremia (≥10 000 copies/ml)

We then classified the study population into three groups; high peak BKV ≥10 000 copies/ml (n = 75), low peak BKV <10 000 copies/ml (n = 88), and the BKV negative group (n = 446). The three groups were compared according to patient and graft survival, BCAR after BKV, mean creatinine and eGFR at 3, 6, and 12 months post-transplant, and the occurrence of BKVAN. There was no statistically significant difference in patient or graft survival among the three groups, P = 0.11, Table 3. The high-viremia group ≥10 000 copies/ml was found to have a statistically significant higher creatinine and lower eGFR at 6 (P = 0.014, P = 0.004, respectively) and 12 (P = 0.01, P = 0.002,

Table 3. Clinical significance of high and low BK viremia, copies/ml.

		Low viremia (n = 88)	High viremia (n = 75)	
	No viremia (n = 446)	<10 000 copies/ml	≥10 000 copies/ml	P-value
Cr 3 months after Tx (mean/SD)	1.52 ± 0.67	1.54 ± 0.5[a]	1.611 ± 0.59[b]	[a]0.32 [b]0.08
eGFR 3 months after Tx (mean/SD)	52.9 ± 19.3	49.77 ± 16.72[a]	48.9 ± 16.6[b]	[a]0.18 [b]0.08
Cr 6 months after Tx (mean/SD)	1.54 ± 0.7	1.6 ± 0.6[a]	1.67 ± 0.58[b]	[a]0.27 [b]0.014
eGFR 6 months after Tx (mean/SD)	52.36 ± 19.9	48.5 ± 16.4[a]	46.4 ± 15.42[b]	[a]0.12 [b]0.004
Cr 12 months after Tx (mean/SD)	1.53 ± 0.7	1.611 ± 0.7[a]	1.69 ± 0.6[b]	[a]0.4 [b]0.01
eGFR 12 months after Tx (mean/SD)	52.26 ± 20	49.5 ± 18.7[a]	45.98 ± 16.1[b]	[a]0.18 [b]0.002
Rejection rate after BKV detected	50/446 (11%)	2/88 (3%)	10/75 (16%)	0.02
Patient survival rate after BKV detected	434/446 (96%)	82/88 (93%)	71/75 (94%)	0.11
Graft loss rate after BKV detected (graft failure and/or death)	29/446 (Death 12 + graft failure 17) 6.5%	11/88 (Death 6 + graft failure 5) 12%	4/75 (Death 4 + graft failure 0) 5.30%	0.11
BKVAN	0/446	0/88	8/75	0.001

The P values refer to the values with the related superscript a or b.
BKV, BK virus; BKVAN, BK viral associated nephropathy; Cr, serum creatinine; Tx, transplant; eGFR, estimated glomerular filtration rate.

respectively) months after transplant when compared with the no viremia group. The low-viremia group <10 000 copies/ml did not have a significant effect on creatinine or eGFR at 6 ($P = 0.27$, 012) or 12 months ($P = 0.4$, 0.18) when compared with the BKV-negative group. The rate of BCAR after BK viremia was initially detected and was significantly higher in the high-peak viremia group compared with the low-peak BK viremia and the BKV-negative groups, $P = 0.02$.

BKV-associated nephropathy

Among the 163 recipients with BK viremia detected at any time, there were 5% (8) that developed BKVAN confirmed by histology, of which 75% (6) were detected in the first year. The overall incidence of BKVAN in the entire screened population was 1.3% (8/609). The median peak (copies/ml) for the eight patients that developed BKVAN were 642 000 (IQR 10th 56 000–90th 4 985 000) compared with the 155 who did not develop BKVAN 5560 (IQR 10th 500–90th 176 800), $P = 0.001$. In addition, the median copies/ml of BKV at the onset of diagnosis were 204 250 (IQR 10th 3500–90th 4 985 000) compared with 2000 (IQR 10th 500–90th 32 400) for those with BK viremia that did not develop BKVAN ($P = 0.009$). Of the 609 patients screened for BKV, 93% (568) had protocol or for cause transplant renal biopsies, and the incidence of occult BKVAN (without BK viremia) was zero.

The characteristics of those with BKVAN ($n = 8$) included seven males/one female; mean age (48 ± 15 years); mean BMI (30 ± 7); five white and three black; three ESRD from diabetes, two hypertension, and three other; six had kidney-only and two had kidney-pancreas transplants; five DD and three living donor (LD); three re-transplants; five had prior dialysis; six were CMV IgG seropositive; and the PRA at transplant (mean 23 ± 32). The time to first BKV detection was mean 7.6 (range 1.4–25.7) months; and the time to diagnosis of BKVAN was 9.1 ± 7 months. The eight BKVAN patients were treated with reduction in IS drugs ($n = 1$), reduction in IS drugs + leflunomide ($n = 5$), and reduction in IS drugs + leflunomide + ciprofloxacin ($n = 2$). After 12–66 months follow-up, seven were alive with stable graft function, and one died with graft function at 11 months from mucormycosis. Those with BKVAN had worse renal function than the other groups ($P < 0.01$), Table 4.

Prediction of BKVAN

Receiver operating characteristic analysis using logistic regression was performed to predict the occurrence of BKVAN using the first positive BKV and the peak BKV viral loads. We found that >185 000 copies/ml – at the time of the first positive BKV diagnosis – to be the strongest predictor for BKVAN with 97% specificity and 75% sensitivity (OR: 113.25, 95% CI: 17.22–744.6, $P \leq 0.001$), Fig. 2a. In addition, the BKV peak viral loads reaching 223 000 copies/ml at any time was found to be predictive for BKVAN with 91% specificity and 88% sensitivity (OR: 70.5, 95% CI: 8.08–615, $P = 0.0001$), Fig. 2b.

Treatment of BK viremia

The 130 recipients with initial BK viremia detected in the first year were treated by one of five different strategies as described in Table 5. The slopes of decline of BK viral loads in the five different treatment groups were compared over the subsequent 2 years or last follow-up after the initial diagnosis. A plot for mean log BKV copies versus time by treatment group was given in Fig. 3. The response variable in this model is the log BKV copies; the risk factors are time, treatment groups, and interaction between time and treatment groups. While those with higher peak BK viral loads were more represented in Groups 2–5, there was no statistically significant difference in the slopes, $P = 0.160$, describing similar rates of viral clearance for each treatment group.

Factors predicting BKV clearance

Factors associated with BK viral clearance were as follows: lower peak viral loads ($P < 0.001$), lower viral loads at first detection ($P = 0.021$), nondoubling of the BK viral load

Table 4. Outcomes for BK viremia and BKVAN.

	No BKV viremia ($n = 446$)	BKV viremia ($n = 155$)	BKVAN ($n = 8$)	*P*-value
SCr 12 months post-Tx	1.5 ± 0.7	1.6 ± 0.6	2.1 ± 0.7	0.02
eGFR 12 months post-Tx	52.3 ± 19.9	48.3 ± 17.6	39.02 ± 14.3	0.01
Treated BCAR	50 (11.2%)	19 (12.2%)	2 (25%)	0.46
Patient survival	434 (97.3%)	145 (93.5%)	7 (87.5%)	0.07
Graft survival	417 (93.5%)	140 (90.3%)	7 (87.5%)	0.36
Death censored graft survival	429 (96.1%)	150 (96.7%)	8 (100%)	0.8

BKVAN, BK virus associated nephropathy; SCr, serum creatinine (mg/dl); eGFR, estimated glomerular filtration rate (ml/min/1.73 m^2); BCAR, biopsy confirmed acute rejection.

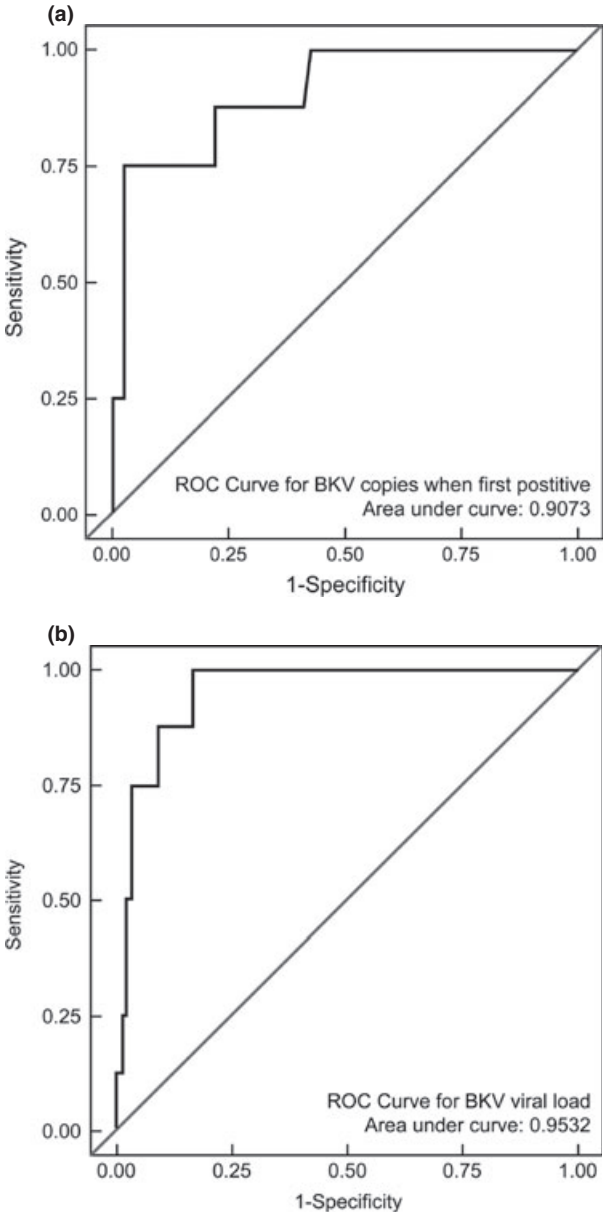

Figure 2 (a) Receiver operating characteristic curve to predict BKVAN when BK viremia (copies/ml) was first detected. Significant cut-off 185 000 copies/ml; with 97% specificity and 75% sensitivity (OR: 113.25, 95% CI: 17.22–744.6, $P < 0.001$). (b) Receiver operating characteristic curve to predict BKVAN from peak BK viremia (copies/ml). Significant cut-off 223 000 copies/ml with 91% specificity and 88% sensitivity (OR: 70.5, 95% CI: 8.08–615, $P = 0.0001$). BKVAN, BK virus-associated nephropathy.

compared with first detection ($P < 0.001$, HR = 5.5, 95% CI: 3.2–9.6), recipient female gender ($P = 0.007$, HR = 1.6, 95% CI: 0.4–0.9), and recipient CMV seronegativity ($P = 0.04$, HR = 1.6, 95% CI: 0.4–0.9). In addition, any 10 000 copies/ml increase in BK viral load from the time of first detection decreased the chance of clearance by 50% ($P = 0.038$, HR = 0.499, 95% CI: 0.3–0.96).

Discussion

BK virus infection is a potential threat to kidney graft function and progression to BKVAN may result in graft loss [1,5,21,22]. A greater awareness of the virus and better understanding of its behavior has resulted in a diminished incidence of BKVAN graft failure from 80% to 15% over the past 20 years [5,6,11,21–23]. The incidence of BK viremia in our study was 26% (at any time after transplant) and 21% (first year); similar to recent reports ranging from 13% to 27% [1,15,20,23–25]. However, the reported risk factors for BK viremia have been inconsistent among different populations. Some studies have identified recipient age, African American (AA) recipients, nondiabetics, DDs, males, HLA mismatch, thymoglobulin use, TAC–MMF use, acute rejection, and placement of ureteral stents as risk factors [16,23,26–31]. While in other studies, none of these risk factors were confirmed [24,32,33] with some reporting diabetes as a risk factor for BKV infection [21], AA recipients to be protected against BKV [24,32], and LD versus DD not associated with BKV [1].

In our study, we analyzed risk factors for BKV infection at any time post-transplant, at 1 year, and for BK viremia ≥10 000 copies/ml. We did not find any of the previously mentioned demographic risk factors significant. However, we did find that higher trough blood levels of both TAC and MMF were associated with an increased incidence of BK viremia, Table 2. This was observed using mean values of all levels and the proximate three levels prior to initial detection. This finding supports the concept that greater exposure to immunosuppression increases the risk for BKV infection, and overall immunosuppression may be more important than any individual drug [26]. While therapeutic drug monitoring of C_0 blood levels may not be as precise a tool to measure actual drug exposure as AUC, those recipients with consistently higher levels over longer periods of time appeared at increased risk for BK viremia. We also found that treated BCAR at any time ($P = 0.002$) or the first year ($P = 0.043$) was a significant risk factor for BK viremia ≥10 000 copies/ml. These findings were similar to Sood *et al.*, who reported acute rejection as an independent risk factor for development of BKV infection [32]. This is perhaps because of the augmented immunosuppression, usually high dose steroids, used for treatment of acute rejection.

We analyzed the long-term impact of low- and high-BK viremia on transplant outcomes. We found that low-BK viremia <10 000 copies/ml did not have a significant impact on graft function measured by serum creatinine and eGFR at either 3, 6, or 12 months after transplant. In addi-

Table 5. Immunosuppressive drug doses and blood levels for 130 patients before and after BKV.

	Number of BKV patients	Pre-BKV		Post-BKV	
		Drug dose	Blood level	Drug dose	Blood level
MMF	130	1000–2000 mg/day	2.95 mg/l	0–1000 mg/day	1.98 mg/l
CNI					
Tacrolimus	127	4–10 mg/day	9.73 ng/ml	2–6 mg/day	7.86 ng/ml
CsA	2	100 mg bid	210 ng/ml	50 mg bid	100 ng/ml
None	1				
Sirolimus	1	No change			
Leflunomide	27			40–100 mg/day	43 212 ± 28 943 ng/ml
Cipro	24			250–500 mg/day	

BKV, BK virus; MMF, mycophenolate mofetil; CNI, calcineurin inhibitor.

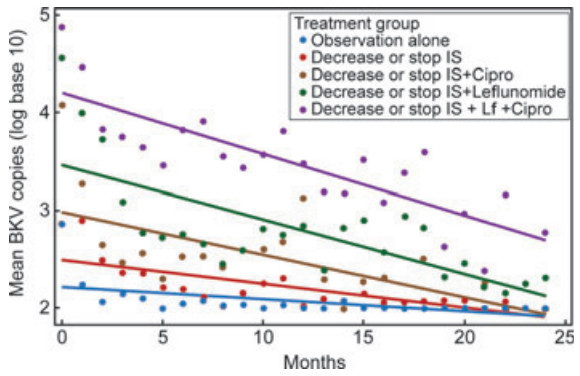

Figure 3 Decline in BK viral loads according to treatment groups (1–5).

tion, initial low-BK viremia did not impose an increased risk to develop acute rejection, graft loss, or BKVAN when compared with the BK-negative group for up to 66 months (Table 3). There is currently no consensus on long-term effect, rate of clearance or treatment strategies for low copy BK viremia. In our population, the spontaneous clearance rate of low-BK viremia was 95% without changing the immunosuppressive protocol or adding antiviral therapy. This finding is in agreement with others, who have reported successful management of low-BK viremia <10 000 copies/ml without reduction in immunosuppression or adding antiviral drugs [25]. Some previous studies have also suggested that BK viral loads <10 000 copies/ml were unlikely to be associated with BKVAN [1,4,11,17]. We also confirmed using protocol kidney biopsies at 3 and 12 months that the incidence of BKVAN in the low-BK viremia group was zero. Thus, these data suggest that close observation is a reasonable option for low-BK viremia <10 000 copies/ml recipients unless increasing viral loads emerge. On the other hand, we found that high viremia ≥10 000 copies/ml is a significant risk factor for graft dysfunction at 6 ($P = 0.004$) and 12 ($P = 0.002$) months after transplant, graft rejection ($P = 0.02$), and BKVAN ($P = 0.001$) when compared with the BKV-negative group. These data suggest that BK viral loads of ≥10 000 copies/ml mandate immediate intervention by planned reduction in immunosuppression.

The incidence of BKVAN in our study population was 1.3% (8/609), 75% (6/8) of cases, the first year with the mean diagnosis at 9 months. Our incidence is lower than several recent reports ranging from 1% to 27% [4,16,20,23,25,34]. We speculate that our relatively low incidence of BKVAN is because of the introduction of PCR surveillance for BKV with rapid reduction in immunosuppression for high-viral load recipients. It is doubtful that cases of BKVAN were missed as patients with and without BK viremia underwent protocol and for cause biopsies at frequent intervals. We also found that 7/8 patients with BKVAN, retained stable, but impaired graft function after 12–66 months of follow-up, further supporting the role for early detection and intervention, Table 4.

The use of BKV copy numbers as a prediction tool for BKVAN can guide clinical management as suggested by several authors [4,17,35–37]. In this study, risk factors for BKVAN were found to be the number of BKV copies/ml at the initial diagnosis of BKV viremia, and at the time of peak viral loads. We found a cut-off value of 185 000 copies/ml at the onset of BKV infection to be the strongest predictor for BKVAN with 97% specificity and 75% sensitivity, Fig. 2a. Moreover, the BKV peak viral loads reaching 223 000 copies/ml were found to be predictive for BKVAN with 91% specificity and 88% sensitivity (Fig. 2b). These levels were somewhat higher than previous studies that considered BK viremia ≥10 000 copies/ml to be most predictive of BKVAN [4,17,38,39]. While all biopsy proven BKVAN patients in our study had BKV loads ≥10 000 copies/ml, the reverse is not true. Only 11% (8/75) of the high-viremia group developed BKVAN. Taking into consideration, the fluctuating behavior of viral loads in the blood [40], we speculate that our higher cut-off values of BK copies/ml might be because of the many samples obtained in

the BK viremic patients, and the close monitoring with histology that both confirmed the diagnosis of BKVAN and minimized the risk of undetected cases. One center reported higher rates of BKVAN than that of our study, but employed much less frequent screening (q 3 monthly), no confirmation of the amount of immunosuppressive reductions, and used a higher target cut-off of 750 compared to our 500 copies [23]. While they found lower rates of overall BK viremia (16% vs. 26%) and higher rates of BKVAN (4.3% vs. 1.3%), these differences are not striking. Our lower rates of BKVAN could reflect our more intense screening and rapid immunosuppressive reductions.

Despite the importance of BK viremia, there is no standardized protocol for managing this infection [25]. The therapeutic strategies for BKV infection in general have been to introduce agents directed against the virus or toward reconstituting the immune system or combination of both. As yet, there is no evidence-based data that defines clinically appropriate target blood levels or size of the reduction in dosages for the immunosuppressive agents commonly used [41]. We have utilized a 30–50% reduction in the doses of the administered agents. Some reports have suggested specific activity against the BKV for cidofovir, leflunomide, intravenous immunoglobulin, and fluoroquinolone antibiotics [23,25,42]. However, the drug cidofovir is nephrotoxic at doses suggested to have anti-BKV activity [4,43,44]. Leflunomide showed modest *in vitro* activity against BKV infection [45], and one study reported decreased viremia and improved histology in 15 of 17 patients converted from mycophenolate mofetil to leflunomide [46]. On the other hand, several studies have questioned the efficacy of leflunomide [47,48] and it has been difficult to separate the antiviral effect of leflunomide from the concomitant reduction in immunosuppression [6]. A potential role for ciprofloxacin in the prevention of BK viremia after kidney transplantation has also been suggested [49]. Although a reduction in immunosuppression alone was reported to be successful therapy for significant BKV infection and BKVAN [50], some have suggested that it may prove insufficient to control polyomavirus replication, or may not be practical in patients at high risk for rejection [4]. Thus, the optimal management for BKV infection has remained elusive. In this study, we investigated whether adding leflunomide and/or cipro to the decreased immunosuppression would add any benefit in terms of viral clearance rates. We found no difference in the rate of viral clearance among the five different treatment protocols, Fig. 3. Specifically, we found no increased viral clearance when leflunomide or cipro were added to a 30–50% reduction in overall IS among high viremia ≥10 000 copies/ml patients. In addition, spontaneous recovery is very likely in terms of low viremia <10 000 copies/ml patients.

Factors that influence BKV clearance are imperfectly defined in the literature [51]. We found that lower BK viral loads at the onset of diagnosis and at the maximum peak of the viral load were significantly associated with viral clearance. This is in accord with Scwarz *et al.*, who reported that the most important influence on viral reduction time was the peak viral load [51]. Moreover, nondoubling of the BK viral load compared with first detection was associated with higher rate of viral clearance ($P < 0.001$, HR = 5.5, 95% CI: 3.2–9.6). In addition, any 10 000 copies/ml increase in BK viral load after the first detection decreased the chance of clearance by 50% ($P = 0.038$, HR = 0.499, 95% CI: 0.3–0.96), which may provide an important role for monitoring viral load kinetics.

While the strengths of our study are the prospective viral screening for all recipients and the routine histological screening of the transplant kidneys, an important limitation was the lack of randomized and blinded treatment selection once BK viremia was detected. In addition, group sizes were probably underpowered to demonstrate narrow treatment differences. As we report a relatively low rate of confirmed BKVAN using our approach, there may be individual patients with BKVAN that demand more intense treatment for viral progression. The primers used in our PCR testing were directed against the large T-antigen gene. Centers that use PCR testing for other viral genome targets may have to validate their own data using their own monitoring tests. Lastly, some have reported that properly performed urine cytology for Decoy cells or urinary-Haufen testing may be useful and cost effective for monitoring recipients with BK infection and/or nephropathy [52,53].

In conclusion, BK viremia is common in the current kidney and kidney-pancreas transplant population, with the majority of cases in the first year (months 2–4). Surveillance for BK viremia can limit the incidence of BKVAN to 1.3%. There are few predicative risk factors for viral loads ≥10 000 copies/ml, other than prior treatment of BCAR and/or higher immunosuppressive drug exposure. Viral loads at diagnosis <10 000 copies/ml can be monitored cautiously, but rising copy numbers demand early reductions in immunosuppressive drug doses of at least 30–50%. Viral loads >185 000 copies/ml at diagnosis or 233 000 at any time were highly predictive of eventual BKVAN. We did not find the addition of leflunomide or ciprofloxacin to result in greater rates of viral clearance.

Authorship

NE, SMF, RA, JS and SBM: designed research study. NE, SMF, XL and DT: collected data. NE, SMF, XL, JS and DT: analyzed data. NE, SMF, JS, TRS, EP, RF, RA and SBM: wrote the article. NE, SMF, JS, TRS, EP, RF, RA and SBM: revised and modified article.

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