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Brief Communication

BK Virus Replication and Nephropathy After Alemtuzumab-Induced Kidney Transplantation

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BK virus nephropathy (BKVN) is a recognized cause of graft failure in kidney transplant recipients. There are limited data on the epidemiology of BK virus (BKV) infection after alemtuzumab induction. By clinical protocol, the kidney transplant recipients at our center were screened with BKV plasma PCR monthly for the first 4 months posttransplant then every 2–3 months for 2 years. A single center retrospective cohort study of all kidney transplant recipients from January 2008 to August 2010 was conducted to determine incidence and outcomes of BKV infection. Descriptive statistics and Kaplan–Meier analysis was performed. Of 666 recipients, 250 (37.5%) developed viruria, 80 (12%) developed viremia and 31 (4.7%) developed BKVN at a median of 17, 21 and 30 weeks, respectively. Induction with alemtuzumab did not significantly affect incidence of BKVN. Increased recipient age, African American race, acute graft rejection and CMV infection were significantly associated with the development of BKVN in multivariate analysis. The incidence of BK viruria, viremia and nephropathy was not significantly different among kidney transplant recipients who received alemtuzumab induction compared to patients receiving less potent induction.

Key words: Alemtuzumab, BK virus nephropathy, kidney transplantation, lymphocyte depletion

Abbreviations: AA, African American; ALM, alemtuzumab; ANC, absolute neutrophil count; ATG, anti thymocyte globulin; BAS, basiliximab; BKV, BK virus; BKVN, BK virus nephropathy; C, Caucasian; CI, confidence interval; CMV, cytomegalovirus; Cr, creatinine; DCD, donation after cardiac death; DNA, deoxyribonucleic acid; EBV, Epstein–Barr virus; ECD, extended criteria donor; FK, Tacrolimus; G-CSF, granulocyte-colony stimulating factor; H, Hispanic; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HLA, human leukocyte antigen; HR, hazard ratio; LEF, Leflunomide; LUKT, living unrelated kidney transplant; MMF, mycophenolate mofetil or mycophenolic acid; PCR, polymerase chain reaction; PRA, panel reactive antibody; SPK, simultaneous pancreas kidney transplant.

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Introduction

BK virus nephropathy (BKVN) is a challenge in the contemporary era of kidney transplantation. Epidemiologic studies estimate that BK viruria, viremia and nephropathy occur in 30%, 13% and 8% of patients at 16, 23 and 28 weeks after renal transplantation, respectively (1). Only 2 of 21 epidemiologic studies have evaluated BKV infection as a primary endpoint in the setting of alemtuzumab induction and these studies were unable to elucidate specific risk factors for BKVN (2,3). As a result, the relative impact of this potent lymphocyte-depleting agent on the epidemiology of BKVN remains poorly defined.

The cellular immune system plays a crucial role in controlling BKV replication. Deficiencies of BK-specific T cell immunity are strongly correlated with the development of BKVN (4). Induction with alemtuzumab, a humanized monoclonal antibody against CD52, induces pan-T cell depletion, which may persist for 2 years posttransplantation (5). Given the importance of cellular immune response on BKVN and the prolonged impairment of this response, transplant patients who receive alemtuzumab would be expected to have an increased incidence of BKVN and/or a more late-onset BKVN. However, previous epidemiologic studies evaluating BKVN as a secondary endpoint after alemtuzumab induction have reported conflicting results (5–9). As our center has one of the largest experiences with alemtuzumab induction for kidney

transplantation, we conducted a single center retrospective cohort study to define the incidence and outcomes of BKV infection.

Methods

BKV monitoring

Since 2008, all kidney transplant recipients have been screened for BKVN by plasma polymerase chain reaction (PCR) monthly for the first 4 months posttransplant, every 2 months for the remainder of the first year, then every 3 months in year 2. Plasma BKV PCR was also performed when recipients had an increase in serum creatinine (Cr) and/or when a kidney biopsy was performed. The performance of urine BKV PCR and the management of patients with detectable BK PCR or BKVN, including changes in immunosuppression, were at the discretion of the treating physician.

Transplant surgery specifics

All kidney and simultaneous pancreas-kidney (SPK) transplant patients received one 30 mg dose of alemtuzumab for induction in addition to a rapid steroid taper, unless they were infected with hepatitis C virus (HCV) or human immunodeficiency virus (HIV) or had a history of cancer with a high risk of recurrence (10). Ureteral stents were used in all of the patients and were removed 6 weeks posttransplant. Patients with allergic reactions to sulfa received atovaquone for 12 months plus ciprofloxacin for urinary tract infection prophylaxis while ureteral stents were in place (see supplement for additional management details).

Protocol kidney biopsies, performed at 3, 12 and 24 months posttransplant and for cause, were routinely stained for the SV40 large-T antigen.

Data collection

Following Institutional Review Board approval, we conducted a single center retrospective cohort study of all patients over 18 years of age who received a kidney or multiorgan transplant with a kidney from January 2008 through August 2010. Each patient's electronic medical record was reviewed to collect recipient information including demographics, immunosuppressive medication regimens and rejection episodes. Onsets of BK viruria, BK viremia and BKVN were co-primary endpoints. BK viruria was defined as any detectable urine BKV PCR; BK viremia was defined as any detectable plasma BKV PCR; BKVN was defined by biopsy with SV40 staining consistent with BKV infection. Graft loss, defined as return to dialysis, and death were secondary endpoints.

Statistical analysis

Student's *t*-test, Fisher's exact or Pearson's chi-square tests were performed where appropriate. Univariate analysis was performed using the Kaplan–Meier method with the log-rank test and multivariate analyses were performed using the Cox proportional hazards model. Variables with a p-value < 0.1 in univariate analysis were included in the multivariate model and the hazard ratio estimates were based on simultaneous analysis of all independent variables. Assumption of proportionality was tested using log-minus-log plot and was met. All analyses were conducted using SAS 9.2 (SAS Inc., Cary, NC, USA). A two-sided p-value of <0.05 was considered statistically significant.

Results

Patient demographics

Seven hundred forty-two patients underwent kidney transplantation between January 2008 and August 2010.

Table 1: Baseline characteristics of kidney transplant recipients (N = 666)

Characteristic	Number (%)[1]
Age in years – median (range)	51 (18–84)
Male sex	403 (60.5)
Caucasian	361 (54.2)
Listing diagnosis	
Diabetes mellitus	229 (34.4)
Hypertension	148 (22.2)
Deceased donor	282 (42.3)
Donation after cardiac death	40 (14.2)
Extended criteria donor	67 (27.8)
Multi-organ transplant	77 (11.6)
HLA mismatches – mean ± SD	3.7 ± 1.7
Desensitization[2] pretransplant	110 (16.5)
Induction therapy	
Alemtuzumab	537 (80.6)
Basiliximab	81 (12.2)
Steroid alone	42 (6.3)
Antithymocyte globulin	4 (0.6)
Maintenance regimen of tacrolimus and MMF	628 (94.3)
Maintenance regimen including prednisone	192 (28.8)
Antirejection treatment	193 (29)
Methylprednisolone	180 (93.3)
Antithymocyte globulin	40 (20.7)
Cytomegalovirus infection	68 (10.2)

[1] Unless otherwise noted.

[2] Desensitization with either rituximab alone or rituximab/IVIG/plasmapheresis for presence of donor specific antibodies, elevated panel reactive antibody (PRA) or ABO incompatibility.

After excluding patients without any BKV screening performed at the time of the chart review (n = 70), who died within 1 month of transplant (n = 1), and who received a combined stem cell-kidney transplant (n = 5), 666 patients were included (Table 1), 80.6% (537) of whom received alemtuzumab induction. Adherence to our screening protocol was poor, with a median of 3 (range 0–32) of the expected 8 BKV PCR results available per patient. There was no difference in protocol adherence between patients who did and did not receive alemtuzumab (median plasma BKV PCR: 3 [0–28] vs. 3 [0–32]). Thirty-five patients did not have BKV PCR sent until after the first year posttransplant. Recipients adhered to the kidney graft biopsy protocol approximately 70% of the time, without noted difference between risk groups.

Frequency of BKV-related endpoints

Two hundred fifty patients (37.5%) developed BK viruria at a median of 16.7 weeks (range 0.1–115.7 weeks) posttransplant, 80 patients (12%) developed BK viremia at a median of 21.1 weeks (range 4.1–108.7 weeks) and 31 patients (4.7%) developed biopsy-proven BKVN at a median of 29.9 weeks (range 8.9–91 weeks; Table 2). Of the 80 patients who developed viremia, 3 did not have urine assessed for viruria. Of the 31 patients who developed BKVN, 1 did not have BKV detected by urine or plasma PCR. Fifteen (48.4%) of the 31 BKVN cases were diagnosed

Table 2: Characteristics of patients by disease state

	No replication n = 412	BK Viruria n = 250		BK Viremia n = 80		BKVN n = 31	
		Value	p-Value[1]	Value	p-Value[1]	Value	p-Value[1]
Recipient age	49.5 (13.6)	50 (12.6)	0.68	51.8 (11.5)	0.122	55.1 (10.1)	0.006
Donor age	40.8 (13.7)	41.2 (14.9)	0.722	41.4 (15)	0.734	44.8 (18.3)	0.237
Male sex	243 (59%)	157 (62.8%)	0.413	54 (67.5%)	0.171	24 (77.4%)	0.056
Donor male sex	197 (47.8%)	127 (50.8%)	0.522	39 (48.8%)	0.903	13 (41.9%)	0.579
AA race	97 (23.4%)	64 (25.6%)	0.641	21 (26.3%)	0.668	14 (45.2%)	0.011
Donor AA race	79 (19.2%)	49 (19.6%)	1	18 (22.5%)	0.539	10 (32.3%)	0.101
Prior transplant	67 (16.3%)	40 (16%)	0.914	10 (12.5%)	0.409	2 (6.5%)	0.2
Prior BKVN	1 (0.2%)	4 (1.6%)	0.071	2 (2.5%)	0.07	0	1
Diabetes mellitus	191 (46.4%)	103 (41.2%)	0.197	34 (42.5%)	0.542	13 (41.9%)	0.710
Chronic viral infection[2]	32 (7.8%)	24 (9.6%)	0.472	6 (7.5%)	0.834	3 (9.7%)	0.739
Multiorgan Transplant	39 (9.5%)	37 (14.8%)	0.045	12 (15%)	0.159	7 (22.6%)	0.031
Deceased donor	170 (41.3%)	112 (44.8%)	0.418	39 (48.8%)	0.219	19 (61.3%)	0.038
Cold ischemic time	17.9 (6)	16.7 (5.9)	0.099	17 (5.5)	0.402	18 (5.9)	0.957
DCD	26 (15.3%)	14 (12.5%)	0.74	4 (10.3%)	0.802	0	0.242
ECD	38 (22.4%)	28 (25%)	0.504	11 (28.2%)	0.222	8 (42.1%)	0.009
DGF	43 (10.4%)	29 (11.6%)	0.7	8 (10%)	1	5 (16.1%)	0.363
HLA mismatch	3.6 (1.8)	3.7 (1.7)	0.665	4 (1.4)	0.064	4.5 (1.4)	0.002
Desensitization	66 (16%)	45 (18%)	0.4	17 (21.3%)	0.163	5 (16.1%)	0.575
Lymphocyte Depleting Induction	338 (82%)	200 (80%)	0.475	66 (82.5%)	1	25 (80.6%)	1
Steroid use	109 (26.5%)	84 (33.6%)	0.052	27 (33.8%)	0.218	11 (35.5%)	0.294
Rejection[3]	92 (22.3%)	100 (40%)	<0.001	36 (45%)	<0.001	19 (61.3%)	<0.001
Cellular	82 (19.9%)	91 (36.4%)	<0.001	33 (41.3%)	<0.001	18 (58.1%)	<0.001
Humoral	19 (4.6%)	31 (12.4%)	<0.001	8 (10%)	0.062	2 (6.5%)	0.651
CMV infection	35 (8.5%)	31 (12.4%)	0.141	17 (21.3%)	0.001	10 (32.3%)	<0.001
Neutropenia	233 (56.6%)	145 (58%)	0.747	50 (62.5%)	0.387	20 (64.5%)	0.453
GCSF use	148 (35.9%)	106 (42.4%)	0.1	42 (52.5%)	0.006	17 (54.8%)	0.036

[1]Compared to no BKV replication.
[2]Defined as infection with HIV, HBV, HCV or EBV.
[3]Either cellular, humoral or both.
Abbreviations: AA = African American; BKVN = BK virus nephropathy; CMV = cytomegalovirus; ECD = extended criteria donor; GCSF = granulocyte colony stimulating factor; HLA = human leukocyte antigen.

by protocol biopsy. Twenty-one (67.7%) of the BKVN diagnoses were pathologic stage A.

Sustained viruria (two detectable urine BKV PCR at least one week apart) was associated with BK viremia and BKVN ($p < 0.001$ when compared to transient viruria). No patient with transient viruria developed viremia or BKVN and only 1 patient with transient viremia developed BKVN. Sustained viremia was also associated with BKVN development when compared to transient viremia ($p = 0.002$). Sustained viruria had a sensitivity of 90.3%, specificity of 80.2%, positive predictive value of 18.2% and negative predictive value for 99.4% for BKVN. Sustained viremia had a sensitivity of 80.6%, specificity of 95.4%, positive predictive1 value of 46.3% and negative predictive value for 99% for BKVN.

Risk factors for development of BKV disease

Older recipient age ($p = 0.006$), African American race of the recipient ($p = 0.011$), deceased donor kidney transplant ($p = 0.038$), multivisceral transplant (SPK, simultaneous liver-kidney and heart-kidney; $p = 0.031$), extended criteria donor use ($p = 0.009$), high degree of HLA mismatch ($p = 0.002$), use of granulocyte-colony stimulating factor (G-CSF; $p = 0.036$) and CMV infection ($p \leq 0.001$) were all associated with the development of BKVN by univariate analysis (Table 2). Although patients with HIV and HCV received basiliximab induction instead of alemtuzumab, there was not a difference in BKV-related endpoints in patients with and without chronic viral infections (i.e. HIV, hepatitis B virus [HBV], HCV, Epstein–Barr virus [EBV]/posttransplant lymphoproliferative disorder; Table 2).

Recipients with graft rejection episodes had a 17.7% higher rate of BK viruria ($p < 0.001$), 22.7% higher rate of BK viremia ($p < 0.001$) and 39% higher rate of BKVN ($p < 0.001$) (Table 2). When cellular and humoral rejection were evaluated separately, cellular rejection remained significantly associated with BKV endpoints, but humoral rejection was only significantly correlated with viruria (Table 2). Rejection occurred before viruria in 37/100 (37%) cases at a median of 14 (0–66) weeks before. Rejection preceded viremia in 12/36 (33%) cases at a median of 12 (0–50) weeks before. Rejection preceded BKVN in 6/19 (32%) cases at a median of 11 (0–51).

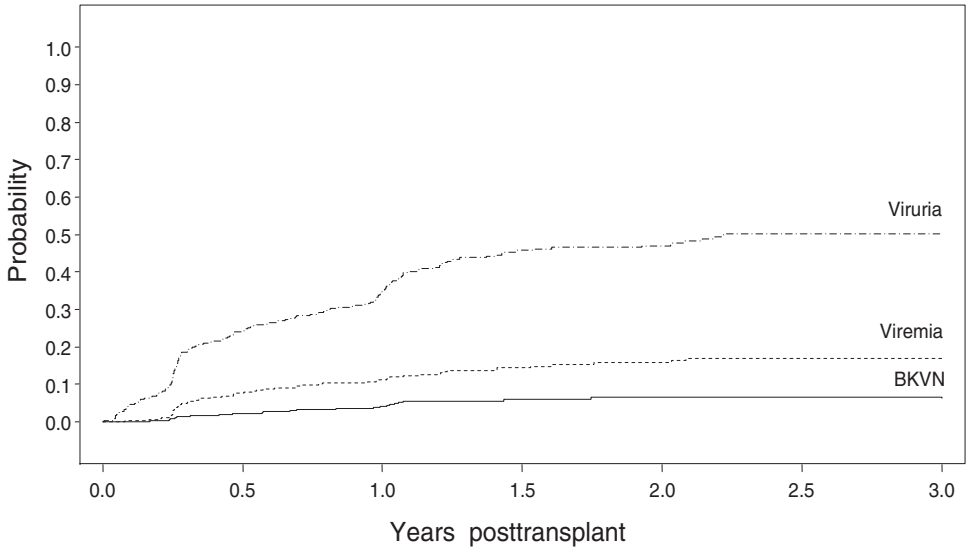


Figure 1: Kaplan–Meier estimates of BK Viruria, Viremia and BKVN. Viral replication was defined by detection of BK DNA by PCR in urine and plasma samples. BKVN was diagnosed by allograft biopsy.

Table 3: Univariate and multivariate models assessing association of patient characteristics with BKVN

	Univariate			Multivariate[1]		
Variable	HR	95% CI	p	HR	95% CI	p
Recipient age	1.04	1.01–1.07	0.020	1.04	1.00–1.07	0.048
Recipient sex	0.46	0.20–1.06	0.067	0.53	0.22–1.27	0.154
AA race	2.58	1.27–5.23	0.009	2.89	1.36–6.14	0.006
Deceased donor	2.53	1.22–5.21	0.012	1.17	0.39–3.53	0.785
Multiorgan	2.35	1.01–5.45	0.047	2.56	0.70–9.33	0.156
ECD	3.83	1.71–8.58	0.001	1.71	0.51–5.78	0.387
Cold ischemic time	1.02	0.95–1.20	0.641	–	–	–
HLA mismatch	1.45	1.12–1.88	0.006	1.21	0.92–1.59	0.165
Steroid	1.36	0.65–2.83	0.418	–	–	–
Rejection	3.46	1.68–7.14	0.001	3.18	1.42–7.12	0.005
CMV infection	3.98	1.87–8.45	<0.001	2.72	1.19–6.24	0.018
GCSF use	1.76	0.87–3.57	0.119	–	–	–

[1]Only variables with $p < 0.1$ in the univariate analysis were included in the multivariate analysis.
Abbreviations: AA = African American; BKVN = BK virus nephropathy; CMV = cytomegalovirus; ECD = extended criteria donor; GCSF = granulocyte colony stimulating factor; HLA = human leukocyte antigen.

Of the 57 patients with sulfa allergies who received ciprofloxacin prophylaxis for the first 6 weeks after transplant, 33.3% developed BK viruria, 3.5% developed BK viremia and no patient developed BKVN. Ciprofloxacin exposure correlated with less BK viremia ($p = 0.049$). Although no patients on ciprofloxacin prophylaxis developed BKVN, this finding did not reach statisticalicance.

Survival analysis

Kaplan–Meier analysis (Figure 1) demonstrates that the 1-year actuarial posttransplant rate of BK viruria, BK viremia and BKVN was 35%, 11% and 4%, respectively. Our Kaplan–Meier estimates of the probability of developing BK viremia and BKVN at 2 years were 16% (95% CI: 12–20%) and 7% (95% CI: 4–10%), respectively, which yielded no statistical difference than what was reported by Hirsch et al. [13% (95% CI: 5–21%) and 8% (95%CI: 1–15%), respectively] (1). Since viruria was measured using decoy cells in the Hirsch study, but measured with PCR in this study, we did not compare our rates of viruria.

In the univariate survival analysis, older age, African American race, deceased donor, multiorgan transplant, ECD donor, HLA mismatch, rejection and CMV infection were all associated with greater hazard of developing BKVN. However, only increased recipient age (HR 1.04, 95% CI 1.00–1.07, $p = 0.048$), African American recipient race (HR 2.89, 95% CI 1.36–6.14, $p = 0.006$), acute graft rejection (HR 3.18, 95% CI 1.42–7.12, $p = 0.005$) and CMV infection (HR 2.72, 95% CI 1.19–6.24, $p = 0.018$) remained significantly associated with the development of BKVN in the multivariate Cox regression analysis (Table 3).

BKV dynamics

In patients who developed BK viremia, urine BK viral loads were significantly higher (8.7 ± 2.7 vs. 6.5 ± 2.2 $\log_{10}$

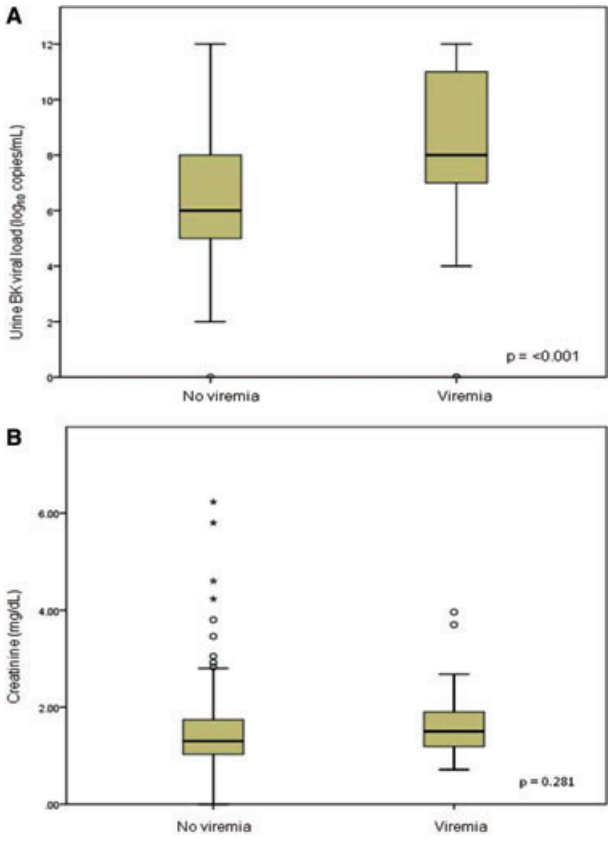


Figure 2: (A) Urine BK viral loads at initial viruria diagnosis in patients with and without BK viremia. (B) Serum Cr at viruria diagnosis in patients with and without BK viremia.

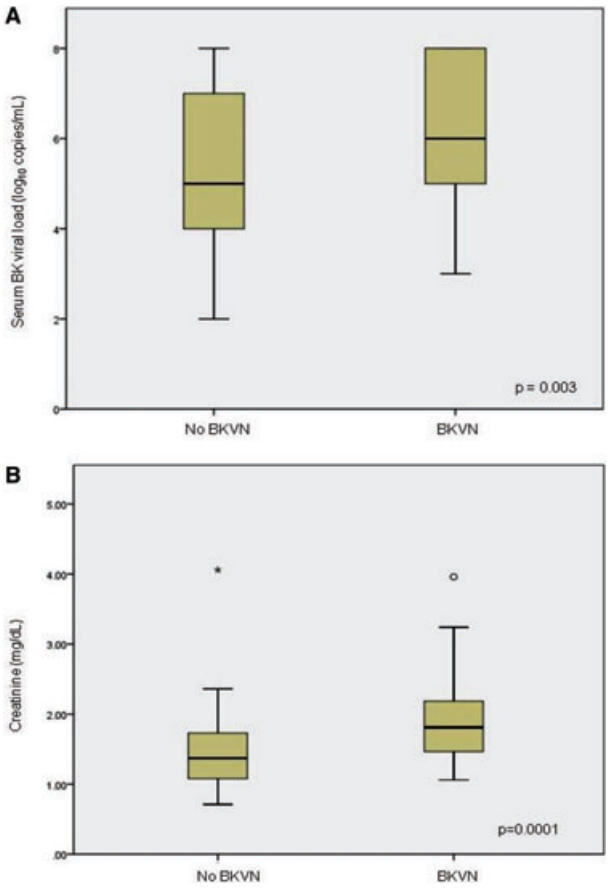


Figure 3: (A) Plasma BK viral loads at initial viremia diagnosis in patients with and without BKVN. (B) Serum Cr at viremia diagnosis in patients with and without BKVN.

copies/mL, p = <0.001), although Cr at viruria diagnosis was not higher (1.6 ± 0.6 mg/dL vs. 1.5 ± 0.9 mg/dL, p = 0.281; Figure 2). In those who developed BKVN, both plasma BK viral loads (6.3 ± 1.5 vs. 5.2 ± 1.7 log_{10} copies/mL, p = 0.003) and Cr at viremia diagnosis were notably higher (2.0 ± 0.7 mg/dL vs. 1.5 ± 0.6 mg/dL, p = 0.001) than those who did not develop BKVN (Figure 3). Mean urine and plasma BK viral loads at histologic BKVN diagnosis were 9.6 ± 2.6 and 5.1 ± 3.1 log_{10} copies/mL, respectively. Mean urine and plasma viral loads in patients who developed BKVN are depicted in Figure 4.

The mean peak plasma viral load was similar in both the alemtuzumab and nonalemtuzumab induced groups (6.2 ± 1.9 vs. 6.9 ± 2.1 log_{10} copies/mL). Furthermore, the time from peak plasma BK viral load to clearance of viremia was not statistically different in these two groups (12.1 ± 12.2 weeks vs. 19.5±20.5 weeks, $p > 0.5$).

BKV treatment

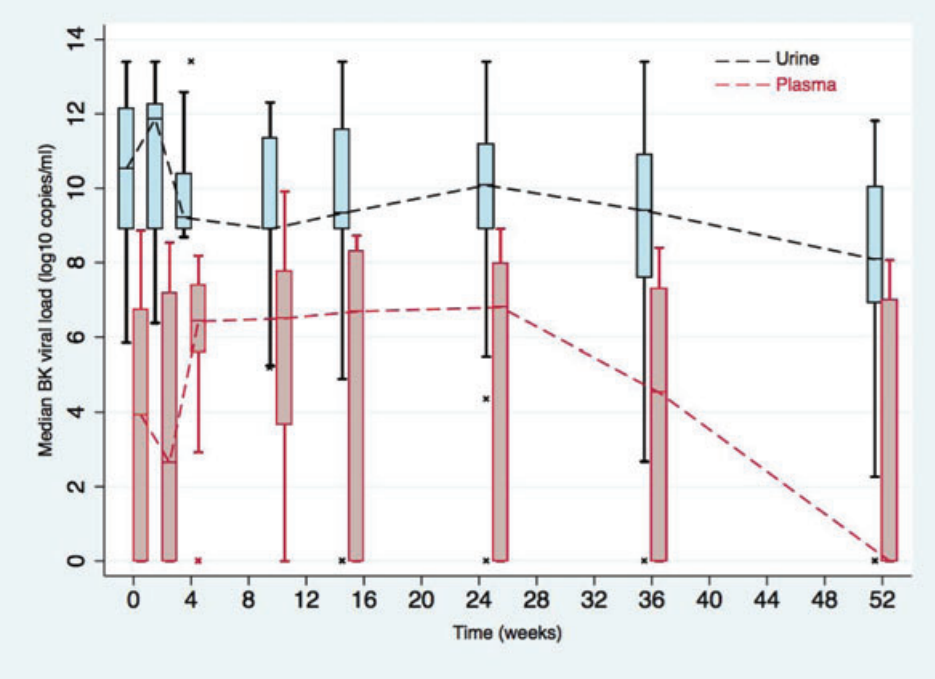
Details about BKV treatment can be found in the supplemental materials.

Patient and graft outcomes

Of the 666 kidney transplant recipients, 38 (5.7%) had kidney graft failure (6 from BKVN and 11 from rejection) and 27 (4.1%) died from an unrelated cause (59.3% with a functioning graft; see Table 4). Of the 31 patients who developed BKVN during the study period, 6 (19.4%) lost their graft due to BKVN. BK viremia (23.7% vs. 11.3%, p = 0.036), BKVN (21.1% vs. 3.7%, $p \leq 0.001$), rejection (68.4% vs. 26.6%, $p \leq 0.001$) and CMV infection (34.2% vs. 8.8%, $p \leq 0.001$) were more common among patients who lost graft function. The course of the six patients with graft loss due to BKVN is outlined in more detail in Table 5. Kaplan–Meier analysis in Figure 5 illustrates the significantly decreased graft survival in patients with BKVN as compared to patients without BKV replication, and those with BK viruria or viremia alone ($\chi^2 = 12.01$, p = 0.0074).

Discussion

This is the largest study to primarily describe the epidemiology of BKVN in alemtuzumab-induced kidney



*Median time of BKVN diagnosis by biopsy was 29.9 weeks (range 8.9 -91 weeks)
** Box-whisper plot depicts medians and inter-quartile (IQR) distributions; whiskers ascribe data within 1.5 x IQR from the nearest quartile

Figure 4: Mean urine and plasma BK viral loads in patients who developed BKVN.

Table 4: Patient and graft outcomes

	All patients (n = 666)	No replication (n = 412)	Viruria (n = 250)	Viremia (n = 80)	BKVN (n = 31)
Graft failure due to BKVN[1]	6 (0.9%)	NA	6 (2.4%)	6 (7.5%)	6 (19.4%)
Graft failure due to rejection[1]	11 (1.7%)	6 (1.5%)	5 (2.4%)	2 (2.5%)	2 (6.5%)
Graft failure due to other causes[2]	22 (3.3%)	17 (4.1%)	5 (2.4%)	2 (2.5%)	1 (3.2%)
Death[3]	27 (4.1%)	18 (4.4%)	9 (3.6%)	3 (3.8%)	1 (3.2%)
Cr at last visit (mg/dL);	1.33	1.32	1.36	1.50	1.87
Median (range)	(0.34–24.7)	(0.34–24.7)	(0.61–10.4)	(0.61–10.4)	(0.67–10.4)
Duration follow-up (days); median (range)	415 (20–1167)	386 (20–1167)	512 (21–1159)	507.5 (90–1060)	495 (221–966)

[1]Etiology of graft loss was clinically defined by the transplant team at the time of allograft loss.
[2]Causes of graft failure: sepsis/multi-system organ failure (6), focal segmental glomerulosclerosis (5), acute tubular necrosis (3), other local infection (3), glomerulonephritis (2), amyloidosis (1), hemolytic uremic syndrome (1), unknown (1).
[3]Causes of death: sepsis (7), unknown (6), cytomegalovirus infection (3), pneumonia (2), malignancy (2), pulmonary embolus (1), cryptococcal meningitis (1), respiratory failure (1), liver failure (1), congestive heart failure (1), infection not specified (1), renal failure (1).

recipients. Among our patients, over a third (37.1%) developed BK viruria after 4 months posttransplant, 12% developed BK viremia after 5 months and 5% developed biopsy-proven BKVN at 7 months. Despite differences in induction, nearly all of our patients received tacrolimus and MMF with similar trough levels for maintenance immune suppression, providing greater homogeneity in our population than in other study cohorts, strengthening the validity of our findings. In the available studies evaluating alemtuzumab induction in adult kidney transplantation, results have been conflicting as to whether alemtuzumab is associated with increased risk of the development of BKVN (2,3,5–9,11–13; Table 6). BKVN was usually a secondary endpoint of these studies.

We found a similar incidence and onset of BKV disease as previous studies that included no or few patients with alemtuzumab induction (1,14). Specifically, our findings are similar to those reported by Hirsch and colleagues in their sentinel paper of BKV epidemiology despite only five

Table 5: Six patients with graft loss due to BKVN[1]

	Age	Race	Type of transplant	Induction	Maintenance	Rejection	Viruria onset (weeks)	Viremia onset (weeks)	BKVN onset (weeks)	Cr at BKVN (mg/dL)	Plasma BK viral load[2] at BKVN	BKVN Rx	Onset of graft loss (weeks)
1	57	AA	Deceased/ECD	BAS	FK/MMF	Yes	8.6	8.6	8.9	3.64	8	IS dec, CID	42.6
2	52	AA	Deceased/ECD	ALM	FK/MMF	Yes	5	14.6	16.3	5.49	8	IS dec, CID	40
3	39	C	SPK	ALM	FK/MMF	No	13	13	24.1	1.91	8	IS dec, CID	35.7
4	44	AA	Deceased	ALM	FK/MMF/Pred	Yes	14.4	29.9	29.9	1.63	8	IS dec, CID, LEF	63.6
5	59	AA	Deceased/ECD	ALM	FK/MMF	Yes	24.4	24.4	26.3	2.38	7	IS dec, CID	42.3
6	44	H	LUKT	ALM	FK/MMF/Pred	Yes	9.1	17.9	32.6	2.13	7	IS dec, CID	64.7

[1] Etiology for graft loss was defined by the transplant team using the patient and graft-specific information; Repeat graft biopsies performed a median of 31 days (0–201 days) before graft loss and 4/6 had residual cytopathic effect consistent with BKVN.
[2] $\log_{10}$ copies/mL.
Abbreviations: AA = African American; C = Caucasian; H = Hispanic; ECD = extended criteria donor; LUKT = living unrelated kidney transplant; SPK = simultaneous pancreas kidney transplant; ALM = alemtuzumab; BAS, basiliximab; FK = tacrolimus; MMF = mycophenolate mofetil; Pred = prednisone; Cr = creatinine; CID = cidofovir; IS dec = immunosuppression decreased; LEF = leflunomide.

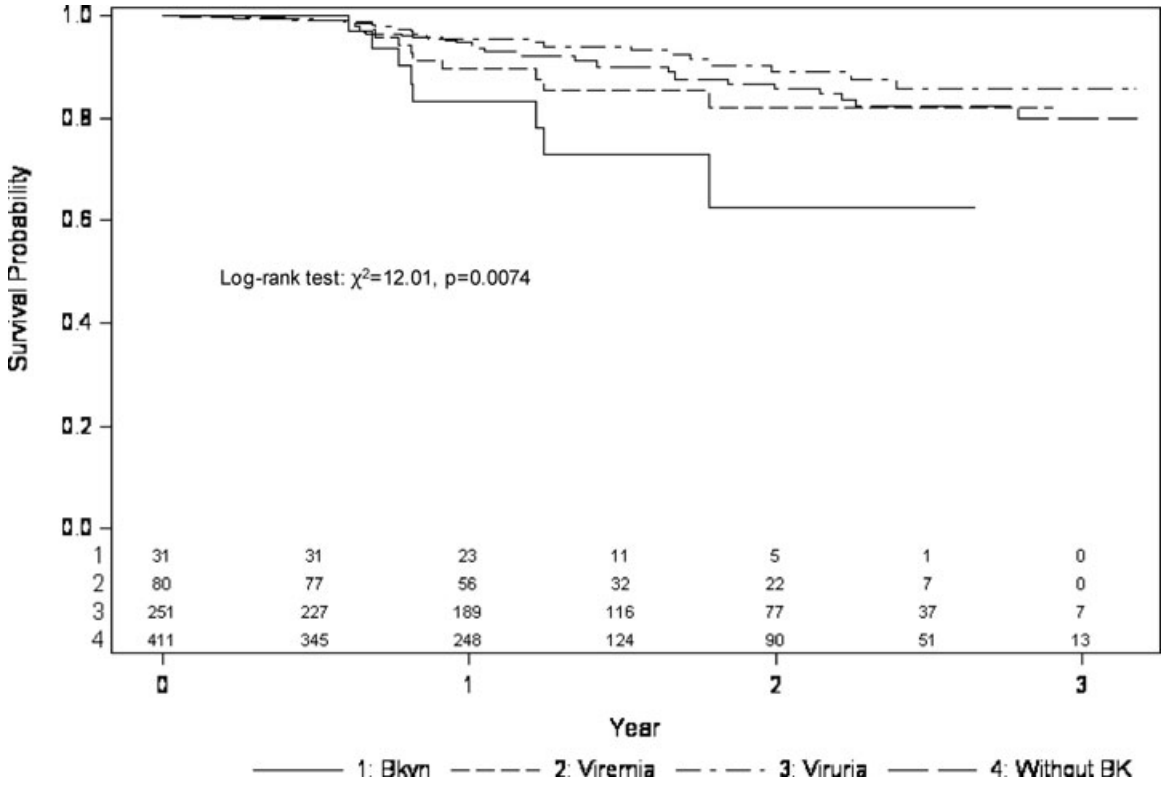


Figure 5: Kaplan–Meier estimates of kidney graft survival.

patients receiving antithymocyte globulin (ATG) and no patient receiving alemtuzumab in the Hirsch cohort (1). Therefore, although we hypothesized that alemtuzumab would delay onset of BKV replication, this does not appear to be the case.

Our data on BKV dynamics support the existing literature, as urine and plasma BK viral loads at BKVN diagnosis were comparable to levels reported in the literature (1,15). In addition, patients who developed viremia had higher levels of virus in their urine and urine viral loads were always higher than those in the plasma (16).

In addition, our data confirms that older recipient age and acute rejection are associated with development of BKVN (1,14). We also found African American recipient race to be associated with a higher rate BKVN without an increased risk for viruria and viremia. A recent prospective study found that African American race protected against the development of BK viruria and viremia, therefore,

Table 6: Summary of studies including adult alemtuzumab-induced kidney transplant patients

Author, year of publication, (reference)	Study type	Study objective	Study group or Comparison	Total patients	Induction regimen	BKV infection
Peleg et al., 2007 (5)[a]	Retrospective cohort	Describe opportunistic infections in patients after ALM induction	ALM induction	547	547 ALM	Viruria: 10 (1.8%) Viremia: 6 (1.1%) BKVN: 1 (0.2%)
Magliocca et al., 2008 (9)[a]	Retrospective cohort	Compare patient death or graft loss in SPK recipients	ALM vs. BAS	331	105 ALM 226 BAS	BKV infection (defined as +urine replication): 4% ALM vs. 4% BAS (p = 1)
Margreiter et al., 2008 (12)[a]	Prospective randomized trial	Evaluate proportion of patients with AR within 6 months	ALM with tacrolimus monotherapy vs. control (triple-drug regimen without induction)	131	65 ALM 66 control	BKV infection (not further defined): 3.1% ALM vs. 1.5% control group
Schadde et al., 2008 (8)[a]	Retrospective cohort	Evaluate outcomes in DCD kidney recipients with differing induction regimens	ALM vs. BAS vs. ATG	170	81 ALM 43 BAS 21 ATG	BKV infection (not further defined): 4.9% ALM vs. 0 BAS vs. 0 ATG (p = 0.07)
Ison et al., 2009 (3)	Retrospective cohort	Describe the incidence and outcomes of SPK recipients with BKVN	ALM vs. ATG	205	146 ALM 59 ATG	BKVN 5-year actuarial rate: 5.1% ALM vs. 6.8% ATG (p = 0.67)
Farney et al., 2009 (6)[a]	Prospective, randomized trial	Evaluate safety and efficacy of ALM vs. ATG	ALM vs. ATG	222	113 ALM 109 ATG	BKVN: 1% ALM vs. 8% rATG (p = 0.01)
Plata-Munoz et al., 2009 (11)[a]	Retrospective cohort	Evaluate graft and patient survival in DCD kidney recipients	ALM vs. BAS	30	15 ALM 15 BAS	BKV infection (not defined): 21% ALM vs. 14% BAS (p = NS)
Tan et al., 2009 (13)[a]	Observational cohort	Describe graft and patient survival in living donor kidney transplant	ALM pretreatment and tacrolimus monotherapy	200	200 ALM	BKVN: 2/200 (1% incidence)
Safdar et al., 2010 (20)[a]	Retrospective cohort	Compare differential risk of all infections in patients receiving different induction regimens	ALM vs. BAS/DAC vs. ATG/ALG	1026	726 ALM 215 BAS/DAC 85 ATG/ALG	BKV infection (defined as +urine or plasma PCR): 14% ALM group (no data in other groups)
Cannon et al., 2011 (2)[b]	Retrospective cohort	Describe the incidence BKV infection after ALM induction	ALM induction	456	456 ALM	BK viremia: 6.6% incidence BKVN: 0.2% incidence

Abbreviations: ALM = alemtuzumab; AR = acute rejection; ATG = antithymocyte globulin; BAS = baxiliximab; DAC = daclizumab; DCD = donation after cardiac death; SPK = simultaneous pancreas -kidney transplant.

[a]Denotes BKV infection was a secondary endpoint of the study.

[b]Only study to evaluate risk factors for BKV infection: Univariate analysis revealed no significant risk factors associated with BK viremia. *Note:* BK viremia increased risk for AR (HR 3.48; 95% CI 1.24–9.76; p = 0.018).

relationship between race and BKV disease is an important element to be included in future research (17). Further, although HLA mismatch was significant in our univariate analysis, it did not remain so after controlling for other factors, including acute rejection. Finally, CMV infection was also significantly associated with the development of BKVN in our cohort, which has rarely been reported in the literature. Since both infections will reactivate in the setting of immune suppression (4), this association may be a marker of the impact that alemtuzumab has on cellular immune responses.

Although graft loss from BKVN ranges widely in the literature (0 to > 50%), aggressive screening combined with reduction of immunosuppression is associated with the best outcomes (14,18,19). The high rate of graft loss attributable to BKVN (6 BKVN-attributed graft loss of 31 (19.4%) patients with BKVN) in this study could be the result of alemtuzumab, compliance with screening, or management choices for patients with BKVN. If alemtuzumab prevented the development of BKV-specific T cell responses, reduction of immunosuppression may fail to control viral replication in our patients. The short interval between detection of BKV in urine or blood and the biopsy-proven diagnosis of BKVN suggests a missed opportunity for early intervention and supports enhancing compliance with our screening protocol (Table 5). Using multiple interventions, we are working to increase compliance with the protocol and will assess the impact this has on detection and outcome of BKVN.

One patient in this cohort was diagnosed with early BKVN by biopsy, but never had a detectable urine or plasma BK viral load despite multiple assays performed. Possible reasons for this anomaly include nephropathy from JC virus, false positive pathology examination of the kidney biopsy or false negative PCR. There was no intervention after the BKVN diagnosis and the patient remained with a stable Cr; therefore, JC virus nephropathy is unlikely.

Our study does have limitations. First, it is a retrospective study, which allows us to only assess associations and not prove causation. As with all retrospective studies, there is the possibility for confounding variables that are not accounted for by our analyses. Second, the comparator group of patients receiving nonlymphocyte depleting induction therapy was small (~20% of our population); we include our non-alemtuzumab-induced patients in this study as a point-of-reference only. Similarly, since our protocol generally defined which patients would not receive alemtuzumab, bias may have been introduced. We evaluated the effect of chronic viral infection and multiorgan transplantation on BKV endpoints in an effort to address this potential for bias. Third, some of the findings could be center-specific since this is a single center study. Fourth, patients generally had few screening plasma BK viral load measurements suggesting that screening may have been incomplete, which limits our ability to make strong conclusions regarding timing and duration of BK viral replication. BKV PCR was usually performed at our in-hospital laboratory, but sometimes performed at an outside lab. As BKV PCR assays are not standardized, it remains difficult to compare BK viral loads between patients and between studies. Finally, BKV treatment was not standardized, so we cannot make strong assessments about the specific role of any of these interventions in the management of BKVN.

In conclusion, this is the largest study primarily focused on the epidemiology of BKV-related disease in alemtuzumab-induced kidney transplant patients. These data suggest that alemtuzumab does not increase rates of BK viruria, viremia or BKVN and does not delay onset of BKV reactivation. The relatively high rate of graft loss in this study suggests that the optimal management of BKVN in alemtuzumab-induced patients, as in other patient populations, has yet to be defined; more frequent screening may be needed in this population. A prospective study to specifically compare BKV-related outcomes in kidney transplant patients receiving lymphocyte-depleting induction therapy versus nonlymphocyte depleting therapies should be performed. In addition, studies are needed to optimize the management of BKVN to further improve outcomes of patients with this disease.

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Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

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Supplemental Results