MAJOR ARTICLE

Relationship of Pretransplantation Polyoma BK Virus Serologic Findings and BK Viral Reactivation after Hematopoietic Stem Cell Transplantation

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Background. Reactivation of polyoma BK virus (BKV) infection is consistently associated with hemorrhagic cystitis in persons who undergo hematopoietic stem cell transplantation (HSCT). In this study, we examined the relationship of reactivation of BKV infection with pre-HSCT serologic findings of BKV antibody.

Methods. Serial urine samples ($n = 1118$) obtained from 140 HSCT recipients were prospectively obtained, and BKV loads were quantified by quantitative polymerase chain reaction. Pre-HSCT anti-BKV immunoglobulin G (IgG) levels were determined by indirect immunofluorescence.

Results. In 68 patients, there was significant peaking (i.e., ⩾3-log increase) in the urine BKV load (median peak, 1.7×10^9 copies/mL; range, 1.1×10^4 to 3.2×10^{14} copies/mL) occurring at a median time of 24.5 days (range, 7–49 days). In 72 patients, low-level BKV viruria occurred without peaking (median BKV load, 10 copies/mL; range, 9.9×10^3 to 1.2×10^{10} copies/mL) at a median time of 24.5 days (range, 7–49 days). Pre-HSCT anti-BKV IgG was positively related to elevated urine BKV load during HSCT ($P < .001$). Binary logistic regression revealed that pre-HSCT anti-BKV IgG level was the only statistically significant factor ($P = .009$) to be associated with a ⩾3-log increase in the peak urine BKV load (positive and negative predictive values, 69% and 68%, respectively). Nine patients developed hemorrhagic cystitis at a median of 56 days (range, 29–160); 7 of these patients were evaluable and were found to have a ⩾3-log increase in the peak BKV load. In binary logistic regression, peaking of the urine BKV load ($P = .026$) and graft-versus-host disease ($P = .033$) were found to be statistically significant risks for hemorrhagic cystitis.

Conclusions. The identification of the serologic status of BKV as a significant risk factor for BKV viruria suggests that it should be included as an integral part of the pre-HSCT evaluation.

Polyoma BK virus (BKV) is a nonenveloped virus that contains circular double-stranded DNA of ~5 kb in size [1]. It is ubiquitous, and primary infection is largely asymptomatic and occurs mostly during childhood. Thereafter, the virus remains latent and innocuous, predominantly in the urinary tract. BKV infection may reactivate during immunosuppression. Diseases in which reactivation of BKV infection may play a pathogenetic role include polyomavirus-associated nephropathy in renal allograft recipients [2] and hemorrhagic cystitis (HC) during hematopoietic stem cell transplantation (HSCT) [3].

HC is a major complication after HSCT. HC before hematopoietic reconstitution is mild and transient. However, cases that occur after engraftment are severe and protracted, requiring repeated bladder irrigation and cystoscopy for hemostasis [4]. The variable severity of HC, in terms of its relationship with the time after HSCT, may be related to different pathogenetic mechanisms [3, 4]. Numerous factors have been etiologically linked to post-HSCT HC, with BKV infection being the most consistently observed [3–6]. Asymptomatic BKV viruria occurred in 5% of healthy individuals and in up to 60% of immunocompromised patients [7]. Furthermore, BKV viruria may also occur in HSCT recipients without HC [3, 4, 7]. Therefore, whether BKV viruria was of causal significance in HC after HSCT remained undefined.

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This problem has partly been resolved by prospective studies that quantify the urine BKV load. Although most HSCT recipients have detectable BKV in the urine, patients with HC excrete significantly higher levels of BKV [8]. Furthermore, peak urine BKV loads consistently precede hematuria [8]. Previous studies from our institution (Queen Mary Hospital; Hong Kong) have shown that these clinical and virologic correlations are highly specific to BKV and have not been observed for other viruses thought to be related to HC, such as adenovirus [8, 9]. However, it has hitherto remained unclear why some patients develop significant increases in the urine BKV load, thus rendering them at risk of developing HC. Neither the BKV genotype [10] nor the type of HSCT [11] were important contributing factors to significant reactivation of BKV infection.

Epidemiologic studies have shown that the BKV seroprevalence is ~50% among persons aged <10 years [12]. In adults, seropositivity rates increase to 70%–90% [12, 13], indicating that most of the population has had past exposure to BKV. However, the relationship between serologic evidence of BKV, BKV viruria, and HC remains unclear [14]. In the present study, we tested the hypothesis that pre-HSCT findings of BKV antibody (anti-BKV) in the blood may identify patients who would develop significant reactivation of BKV infection during HSCT.

MATERIALS AND METHODS

Patients and HSCT protocols. One hundred forty patients undergoing HSCT at Queen Mary Hospital were studied. The study group comprised 61 patients recruited from among 215 individuals who underwent HSCT during the period from August 1999 through December 2001, and 79 patients recruited from among 139 individuals who underwent HSCT during the period from June 2004 through August 2005. Patients who did not consent to the study or whose urine sample collection could not be completed were not included in the study. Because we previously demonstrated that patients who undergo autologous and allogeneic HSCT have similar risks of reactivation of BKV infection, both groups of patients were included in the present study. The clinical and virologic data for 68 patients were reported elsewhere [8, 15]. All patients received standard prophylaxis and treatment for graft-versus-host disease (GVHD) and antimicrobial prophylaxis, including ciprofloxacin ($n = 46$), levofloxacin ($n = 38$), and nonquinolone antibiotics ($n = 56$). Two-mercaptoethane sulfonate (MESNA) was administered to patients receiving cyclophosphamide-containing conditioning regimens. Oral mucositis was evaluated daily by designated nurses with no information on BKV virologic/serologic data, using a standardized form that contained 9 items concerning the patient's voice, swallowing sensation, mucous membrane appearance, and subjective assessment of severity. Scores ranged from 9 (normal) to 36 (most severe). The investigation was approved by the institution review board in accordance with the Declaration of Helsinki.

Diagnosis and treatment of HC. Severe HC was defined as painful gross hematuria (with or without blood clots) not due to bacterial infection and not due to bleeding diathesis [3], and the diagnosis was confirmed endoscopically. All patients with HC in this study required bladder irrigation and cystoscopy for hemostasis.

Urine specimen collection. Serial urine samples were collected prospectively before conditioning, on the day of marrow infusion (day 0), and weekly thereafter until day 50. For 23 patients, 24-h urine samples were collected, and 50 mL of the sample was aliquoted for virologic studies. Subsequent studies showed that the BKV load did not exhibit diurnal variations (authors' unpublished data). Therefore, a spot 50-mL urine sample was collected from the remaining 117 patients. Urine samples were centrifuged at 2000 *g* for 10 min. DNA was extracted from 200 μL of free urine (QIAamp Blood Minikit; Qiagen) and eluted with 200 μL of buffer.

Quantification of urine BKV load and definition of significant peaking. Quantification of the urine BKV load was performed by quantitative PCR (Q-PCR) (ABI Prism 7700 Sequence Detector; PE Biosystems). Sequences of the quantitative PCR primers and TaqMan probe (targeting the BKV *VP1* gene), the plasmid standard containing the targeted BKV *VP1* gene, amplification protocols, PCR precautions, and quality assurance have been reported elsewhere [8, 15]. All experiments were performed in triplicate. The urine BKV load was expressed in BKV genome copies per milliliter of urine. Significant peaking of the urine BKV level was defined as a peak urine BKV level of $\geqslant$3-log the baseline level, because previous studies have demonstrated that this cutoff could best distinguish 2 groups of patients with different risks of severe HC [8, 15].

Quantification of the plasma BKV DNA level. Plasma samples were obtained from HSCT recipients concomitant with the urine samples. Quantification of the plasma BKV DNA level was performed as described elsewhere [8], with results expressed as BKV genome copies per milliliter of plasma. To exclude potential interference from plasma inhibitors, known amounts of the BKV *VP1* plasmid standard were spiked into plasma samples as positive controls, which were included in all experiments.

Indirect immunofluorescence assay. The detection of anti-BKV antibody (IgG) was performed using a standard indirect immunofluorescence method, as described elsewhere [16, 17]. A human embryonic lung fibroblast line was infected with BKV and served as the reagent cells, with uninfected cells used as negative controls. Serum samples obtained from patients or hematopoietic stem cell donors before HSCT were screened at a dilution of 1:10. Cell smears were incubated with the tested serum for 30 min at 37°C, followed by two 5-min washes in

phosphate-buffered saline. Anti–human IgG fluorescein isothiocyanate conjugates (INOVA Diagnostic) were added, and the cells were further incubated for 30 min at 37°C. The specimens were then mounted with buffered glycerine and examined under UV light (Olympus IX70; Olympus Corporation). A positive control with a commercially available anti-BKV antiserum (Vector Laboratories) showed immunostaining localized to the nuclei, with uninfected cells being entirely negative (figure 1). This control was included as quality assurance. Serum samples positive at a 1:10 titer were further titrated with serial 2-fold dilutions until endpoint (negative immunofluorescence). Sequential serum samples obtained from each patient were assayed in the same experiment. The highest dilution to yield a positive result was recorded as the antibody titer.

Statistical analysis. Comparison between groups of data was performed using the Kruskal-Wallis test. Risk factors for reactivation of BKV infection were evaluated by binary logistic regression (SPSS). The occurrence of significant peaking (i.e., a $\geqslant$3-log increase over the baseline value) of the urine BKV load was the dependent variable, with age, sex, sources of hematopoietic stem cells (autologous vs. sibling donors vs. matched, unrelated donors), conditioning regimen (regimens that did not contain busulfan vs. regimens that contained busulfan), and the pre-HSCT anti-BKV IgG serologic findings entered as covariates. The impact of pre-HSCT serologic test results on reactivation of BKV infection was also evaluated by the Pearson χ^2 test. Risk factors for the occurrence of severe HC were similarly evaluated, with the following factors as covariates: age, sex, source of hematopoietic stem cells (autologous vs. sibling donors vs. matched, unrelated donors), conditioning regimen (regimens that did not contain busulfan vs. regimens that contained busulfan), and the occurrence of GVHD. P values $<.05$ were considered to be statistically significant.

RESULTS

Patients. Demographic and clinicopathologic features of the patients are presented in table 1. None of the patients had a history of a previous urinary tract pathology or pelvic irradiation.

Clinicopathologic features of BKV viruria. The urine BKV load was quantified in 1118 samples obtained from 140 patients (median number of samples per patient, 8). Serial BKV level quantification revealed 2 distinct patterns of viruria. In 68 patients, there was significant ($\geqslant$3-log increase) peaking of the urine BKV load during HSCT, with a median peak urine BKV load of 1.7×10^9 (range, 1.1×10^4 to 3.2×10^{14} copies/mL) at a median time of 24.5 days (range, 7–49 days). Interestingly, there was a modest decrease in the urine BKV load on day 21 after HSCT. Similar patterns have been observed previously [15]. In the other 72 patients, there was low-level BKV viruria throughout HSCT without significant peaking, with a median maximum urine BKV load of 9.9×10^3 copies/mL (10 to 1.2×10^{10} copies/mL) (figure 2). In 4 of the patients, the urine level of BKV was <100 copies/mL throughout the course of HSCT. The 2 groups of patients did not differ with regard to age, underlying diagnosis, conditioning regimen, or type of HSCT (table 1).

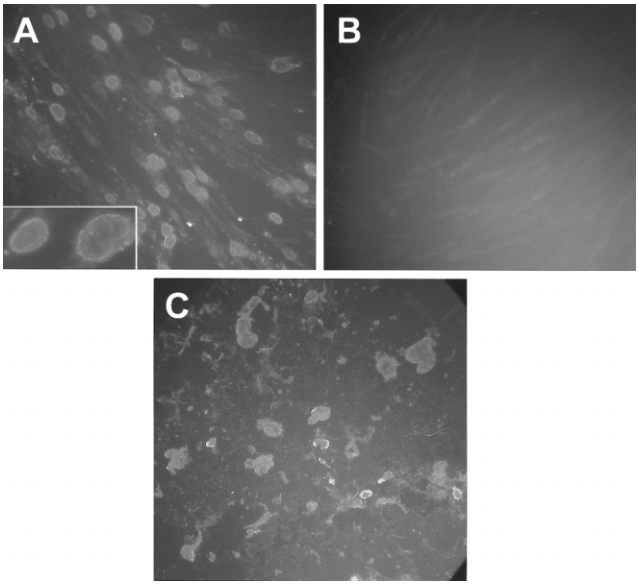

Figure 1. *A,* Positive immunostaining when polyoma BK virus (BKV)–infected human embryonic lung fibroblasts were incubated with an anti-BKV antibody (1:10) as a positive control. Cytopathic effects were demonstrated by the enlargement of nuclei. Cytoplasm was counterstained with Evan's blue stain (which appears red under UV light). *Inset,* Localization of immunostaining to the nuclei of infected cells. *B,* Negative control when uninfected cells were incubated with the anti-BKV antibody at the same dilution. There was no immunostaining in uninfected cells. *C,* Serum from a BKV-seropositive patient showing positive immunostaining of BKV-infected cells. Note that, to increase the test sensitivity, the infected cells were detached from the culture flask and then placed on glass slides before immunostaining, accounting for the somewhat distorted cellular outline.

BKV viruria was not related to BKV viremia. After determining the patterns of BKV viruria, we examined whether BKV viremia behaved similarly. Twenty patients (10 with and 10 without significant peaking of the urine BKV load) were randomly selected for serial measurement of plasma BKV load during HSCT. In all cases, BKV viremia occurred at low levels, with no significant changes throughout HSCT. Quantitative BKV PCR of plasma samples spiked with known amounts of the BKV VP1 plasmid standard (positive control) yielded the expected results, ruling out PCR interference from plasma components (data not shown). Therefore, BKV viremia did not appear to be related to BKV viruria (figure 2).

Mucositis had no effect on BKV viruria. Oral mucositis was used as a surrogate marker for the extent of generalized

Table 1. Clinicopathologic features of and urinary reactivation of polyoma BK virus (BKV) infection among hematopoietic stem cell transplant (HSCT) recipients.

Characteristic	Reactivation of urinary BKV infection		*P*
	Patients with peaking of urine BKV load	Patients with no peaking	
No. of patients	68	72	
Age, median years (range)	42 (21–60)	38 (17–63)	.134
Sex			
Male	39	39	.704
Female	29	33	
Diagnosis			
Acute lymphoblastic leukemia	10	8	.311
Acute myeloid leukemia	22	17	
Chronic myeloid leukemia	11	21	
Myelodysplastic syndrome	2	7	
Lymphoma	14	12	
Myeloma	6	5	
Other	3	2	
Conditioning regimen			
Busulfan and cyclophosphamide	32	40	.502
Cyclophosphamide and total body irradiation	21	18	
Cyclophosphamide, carmustine, and etoposide	8	4	
Fludarabine and total body irradiation	3	6	
Other	4	4	
Source of hematopoietic stem cells			
Autologous	6	5	.909
Allogeneic (myeloablative)			
HLA-identical sibling	33	38	
HLA-matched, unrelated donor	24	21	
Other[a]	2	2	
Allogeneic (nonmyeloablative)	3	6	
Graft-versus-host disease grade			
0	34	46	.238
I–II	23	16	
Greater than II	5	5	

NOTE. Data are no. of patients, unless otherwise indicated. Significant peaking of the urine BKV load viruria was defined as a ⩾3-log increase over the baseline level.

[a] Two patients in each group received HSCTs from parents or siblings with 1 HLA antigen mismatched

mucositis (including the uroepithelium) after HSCT. The mucositis scores (a score of 9 denotes normal presentation, and a score of 36 indicates the most severe presentation) were available from 66 patients, including 31 patients with and 35 patients without significant peaking of the urine BKV load. Among patients without BKV level peaking, the mucositis score increased from a pre-HSCT baseline of 12.71 ± 0.23 to a maximum of 17.8 ± 0.56 at day 14 after HSCT. The corresponding scores for patients with peaking of the urine BKV load were similar (12.9 ± 0.28 and 18.3 ± 0.56, respectively). There was also no significant difference in scores between the 2 groups of patients at any other time points throughout HSCT ($P > .05$, by the Mann-Whitney *U* test).

Pre-HSCT BKV serology of patients was significantly related to post-HSCT BKV viruria. Pre-HSCT serum samples were available for 76 patients. The pre-HSCT anti-BKV IgG titer had no impact on the baseline pre-HSCT BKV load (figure 3*A*). However, anti-BKV IgG had a highly significant correlation with maximum urine BKV load during HSCT (figure 3*B*). The median maximum urine BKV load for patients with a pre-HSCT anti-BKV IgG titer of ⩽1:10 was 2.26×10^3 copies/mL, compared with 2.47×10^8 copies/mL among patients with an anti-BKV IgG titer of ⩾1:20 ($P < .001$). Because most patients had prior exposure to BKV, the seronegative status may have reflected an impaired immune system. To examine whether this was a contributing factor, the serostatus to another ubiquitous

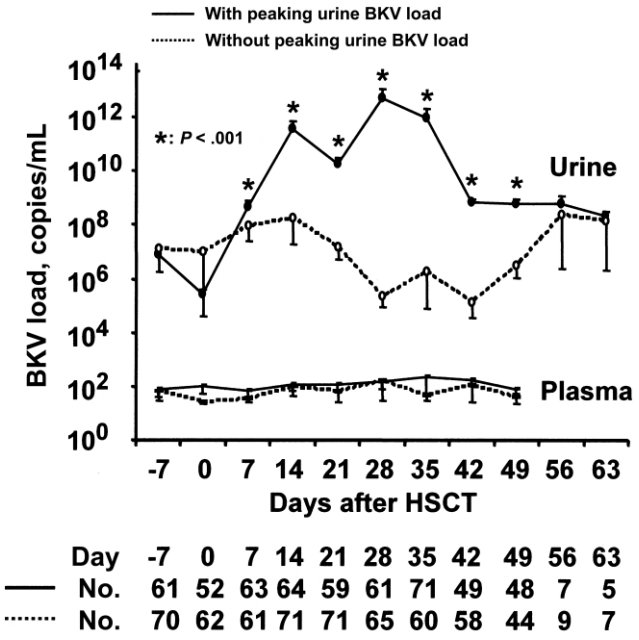

Figure 2. Reactivation of polyoma BK virus (BKV) infection during hematopoietic stem cell transplantation (HSCT). Two patterns of BKV viruria were found, comprising cases that involved significant peaking ($n = 68$) and those without peaking ($n = 72$) ($P < .001$, by the Mann-Whitney U test). Twenty-nine of the 68 patients who had significant peaking and 32 of the 72 patients without peaking have been described elsewhere [15]. When the plasma BKV load was quantified (for 10 randomly selected patients in each group), no detectable changes were found throughout the course of HSCT. Each data point represents the mean ± SEM. No., number of patients included in each time point.

virus (cytomegalovirus) was examined in these patients. There was no significant correlation between pre-HSCT anti-BKV and cytomegalovirus antibody serostatus (37 of 39 patients were positive for anti-BKV and cytomegalovirus antibody, and 31 of 37 were negative for anti-BKV and positive for cytomegalovirus antibody; $P = .115$, by χ^2 test).

Factors predicting a significant peaking of the urine BKV load. When patients were further divided into those with and those without significant peaking of the urine BKV load (defined as a ⩾3-log increase over the baseline value), an anti-BKV IgG of ⩾1:20 was found to be a significant discriminating factor ($P = .001$) (table 2). The sensitivity and specificity of an anti-BKV IgG of ⩾1:20 for significant peaking in the urine BKV load were 69% and 68%, respectively, with positive and negative predictive values of 69% and 68%, respectively. These values were remarkably similar, because the prevalence of peaking of the urine BKV level was 51% among the 76 patients analyzed. Finally, binary logistic regression was performed to assess the predictive value of pre-HSCT serologic findings of anti-BKV. Among other factors, including sex, age, conditioning regimen, type of donor, and GVHD, a pre-HSCT anti-BKV IgG titer of ⩾1:20 was the only significant risk factor ($P = .009$) to predict a ⩾3-log increase in the urine BKV load (table 2).

Donor's BKV serologic findings. We tested for anti-BKV IgG in 57 donors, 32 of whom had a titer of ⩾1:20. The donor's BKV serologic findings did not correlate with BKV viruria in the recipient, with regard to either BKV load or a ⩾3-log increase in the urine BKV load (table 3). Donor BKV serology has no impact on the risk of reactivation of BKV infection among seronegative recipients (data not shown).

Risk factors of severe postengraftment HC. Nine patients developed severe postengraftment HC, at a median of 56 (29–160) days. All received allogeneic myeloablative HSCT. In seven patients, onset of HC was preceded by a ⩾3 log peaking of BKV viruria. In the remaining 2 cases, HC occurred beyond the urine collection period (120, 160 days); thus, the association

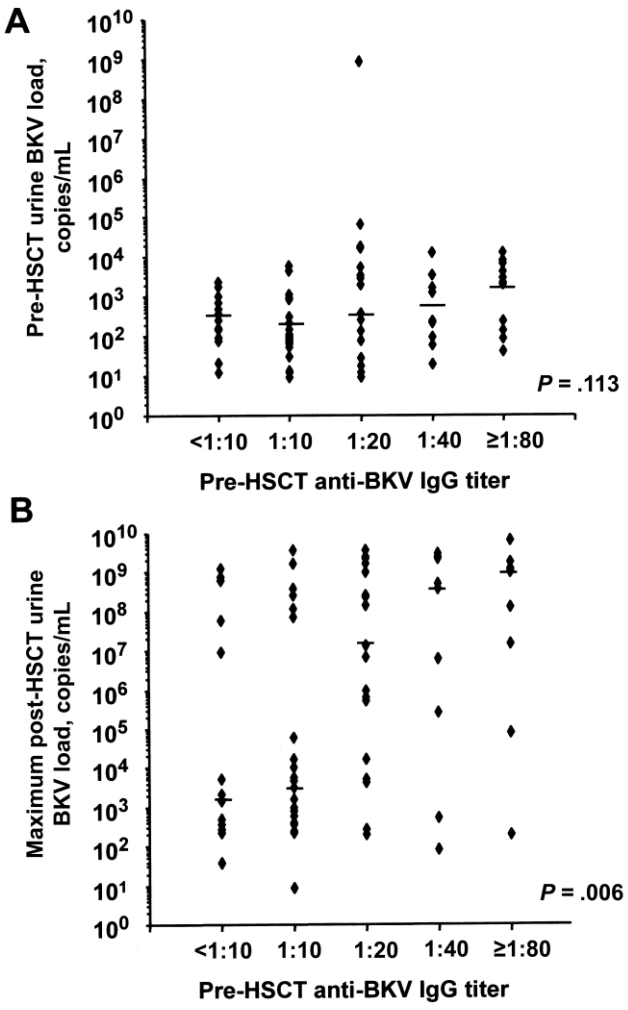

Figure 3. Correlation between pre–hematopoietic stem cell transplant (HSCT) polyoma BK virus (BKV) antibody (anti-BKV) titer and the urine BKV load. Anti-BKV IgG level was unrelated to pre-HSCT urine BKV load *(A)* but was significantly related to the maximum post-HSCT urine BKV load *(B)*. Groups of data were compared using the Kruskal-Wallis test for nonparametric data.

Table 2. Transplant recipients' pretransplantation polyoma BK virus (BKV) serologic analysis and urinalysis findings.

Characteristic	Peaking of urine BKV load		OR (95% CI)	P^a
	<3-log increase over baseline level	≥3-log increase over baseline level		
Pretransplantation BKV antibody IgG titer, no. of patients				
≤1:10	25	12	…	
≥1:20	12	27	…	.001
Potential risk factor for significant peaking of the urine BKV load[b]				
Sex	…	…	0.515 (0.149–1.781)	.295
Age	…	…	1.020 (0.963–1.080)	.497
Serologic test results	…	…	4.543 (1.452–14.212)	.009
Conditioning regimen	…	…	1.665 (0.502–5.528)	.405
Type of donor	…	…	2.544 (0.712–9.089)	.151
Acute GVHD	…	…	2.493 (0.117–53.145)	.558

NOTE. Significant peaking of the urine BKV load viruria was defined as a ≥3-log increase over the baseline level. GVHD, graft-versus-host disease.

[a] Determined using the χ^2 test.

[b] From binary logistic regression analysis.

between BKV viruria and HC could not be ascertained. Binary logistic regression showed that a ≥3 log peaking of BKV viruria ($P = .026$) and GVHD ($P = .033$) were the only 2 significant risk factors for severe HC (table 4).

DISCUSSION

In this report, we investigated factors predisposing to active BKV replication, as reflected by a ≥3-log increase in the urine BKV load from baseline, in a large cohort of HSCT recipients. We showed that pre-HSCT anti-BKV IgG levels were directly related to urine BKV load during HSCT. Furthermore, an anti-BKV IgG titer of ≥1:20 was the only significant risk factor for significant peaking of the urine BKV load in binary regression analysis. These findings might be explained by the tentative hypothesis that higher pre-HSCT anti-BKV IgG levels may reflect a heightened immune response against more active intrinsic BKV infection that is kept under control, so that there is no apparent increase in the urine BK load before transplantation. However, owing to the immunosuppression subsequent to HSCT, uroepithelial BKV replication becomes uncurbed. Patients with more active preexisting BKV replication (reflected by higher anti-BKV IgG) would excrete more BKV in the urine, leading to viruric peaking. Our results corroborated with an early nonquantitative study in which post-HSCT BKV viruria, as detected by ELISA and a DNA hybridization assay, occurred exclusively in BKV-seropositive patients [18]. Interestingly, 10 of 37 patients with a pre-HSCT anti-BKV IgG titer of ≤1:10 exhibited significant BKV viruria during HSCT. These patients may have an attenuated immune response to BKV before HSCT associated with underlying hematologic malignancies or previous chemotherapy, compared with the initial status at time of disease diagnosis; however, it remains possible that some of these patients may have had primary BKV infection rather than reactivation. On the basis of the existing data, we could not distinguish between the 2 possibilities. Moreover, an evaluation of the link between pre-HSCT serologic evidence of BKV and the occurrence of severe HC was also limited by the small number of HC occurring during the study period. Furthermore, during HSCT, patients undergo intense immunosuppression in which both cellular and humoral immunity are suppressed.

Table 3. Transplant donors' polyoma BK virus (BKV) serologic findings and recipients' urine BKV data.

Donor BKV antibody IgG titer	Maximum urine BKV load in recipients		Peaking of urine BKV load in recipients, no. of patients		
	Median copies/mL	P^a	<3-log increase over baseline level	≥3-log increase over baseline level	P^b
≤1:10	3.92×10^6	.873	12	13	.729
≥1:20	1.26×10^8		15	17	

[a] Determined using the Mann-Whitney *U* test.

[b] Determined using the χ^2 test.

Table 4. Binary logistic regression analysis of potential risk factors for postengraftment severe hemorrhagic cystitis.

Potential risk factor	OR (95% CI)	*P*
⩾3-log increase in urine BKV load over baseline level	12.490 (1.353–115.281)	.026
Sex	0.478 (0.072–3.164)	.444
Age	1.002 (0.915–1.096)	.974
Conditioning regimen	7.069 (0.755–66.164)	.087
Acute GVHD	9.986 (1.207–82.625)	.033
Type of donor	0.253 (0.036–1.763)	.165

NOTE. BKV, polyoma BK virus; GVHD: graft-versus-host disease.

Therefore, after transplantation, serologic titers may not accurately reflect the host's response to BKV infection and were not examined in this study.

In the present study, only a small proportion of patients with a ⩾3-log increase in the urine BKV load developed HC. To further delineate other collaborating factors predisposing to HC, we performed regression analysis of a number of potential risks. Microscopic hematuria, which we defined previously as grade 1 HC [4], was not examined in this study, because it was mostly self-limiting and could be confounded by concomitant thrombocytopenia and occult urinary tract infection. The results showed that, in addition to peaking of the urine BKV load, GVHD was a significant risk factor for HC. This is consistent with results of our previous study [4] and of other studies [19], which showed that GVHD is associated with postengraftment HC. This was unlikely to be related to the increased immunosuppression used for the treatment of GVHD, because high-dose corticosteroid therapy for GVHD did not increase the urine BKV load (authors' unpublished results). The results support the immune reconstitution basis of HC after HSCT [15], which was also highlighted by the recent finding that, although patients who underwent autologous and allogeneic HSCT developed similar patterns and magnitude of BKV viruria, severe HC occurred exclusively among allogeneic HSCT recipients [11]. Furthermore, patients who received bone marrow or umbilical cord blood from matched, unrelated donors were at significantly higher risk of HC, compared with recipients of transplants from matched, related donors [20].

We did not observe significant changes in plasma BKV DNA level during HSCT, even in patients who experienced significant peaking of the urine BKV load. This was also found in our previous prospective study of 50 HSCT recipients [8]. Control experiments were carefully conducted to exclude the possibility that plasma inhibitors caused interference with PCR results. Therefore, we reasoned that extension of the serial quantification of plasma BKV load to all patients included in the study would probably yield the same results. The absence of systemic BKV viremia suggests that BKV reactivates only locally in the uroepithelium, the site of latent infection. Our results were different from the results of previous studies, which reported detection of BKV DNA encoding the small-t antigen in plasma samples obtained from HSCT recipients, with levels correlating with the occurrence of postengraftment HC [21, 22]. Whether these discrepant findings may be related to differences in patient populations or assay methods will need to be further examined.

Our findings represent an advancement in the study of BKV in HC after HSCT. Although serial quantification of the urine BKV load is highly accurate for defining the patient population at risk of HC, quantification is not practical without a prospective study. Furthermore, quantification of urine BKV load is labor intensive and is not cost-effective for many HSCT centers. Finally, in most instances, HC occurs shortly after peaking of the urine BKV load, making the timely institution of preventive measures difficult. Our observation of the diagnostic value of anti-BKV IgG offers a potential test before HSCT that might identify patients at high risk of developing significant BKV viruria. The test is especially valuable to the formulation of strategies aiming at suppressing BKV replication, such as the use of quinolone antibiotics [15] or other antiviral agents [7]. From a clinical relevance perspective, because significant BKV viruria will have to partner with GVHD to cause HC, testing for an anti-BKV titer before HSCT should form part of the evaluation of patients who are at high risk of GVHD—especially those who are receiving transplants from matched, unrelated donors. Testing for anti-BKV titer before HSCT will also provide a means to define how modulation of GVHD may impact on the BKV-GVHD collaboration in causing HC.

In conclusion, the identification of serologic evidence of BKV as a significant risk factor for BKV viruria suggests that serologic testing should be included as an integral part of pre-HSCT evaluation. To this end, the development of an ELISA for serologic testing for BKV would provide a more convenient assay for the validation of our observations.

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Potential conflicts of interest. All authors: no conflicts.

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