BK Virus Infection Is Associated with Hematuria and Renal Impairment in Recipients of Allogeneic Hematopoetic Stem Cell Transplants


BK virus (BKV) is an important pathogen and cause of nephropathy in renal transplant recipients, but its significance following hematopoetic stem cell transplantation (HSCT) is less well described. We measured blood and urine BKV in 124 allogeneic HSCT patients (67 had undergone prior HSCT [surveillance cohort]; 57 were monitored from transplant day 0 [prospective cohort]). BK viruria was manifest in 64.8% of the patients; 16.9% developed viremia. In the prospective cohort, the median time from transplantation to BK viremia development (128 days) was longer than for viruria (24 days; P < .0001). Among clinical factors (sex, disease, transplant type, alemtuzumab use, cytomegalovirus [CMV] viremia, graft-versus-host disease [GVHD], donor HLA C7 allele), only CMV viremia was more common in patients with BKV infection (P ≤ .04). There was a direct relationship between blood and urine BKV levels and the occurrence, and degree, of hematuria (P ≤ .03). Finally, BKV infection was analyzed along with other clinical factors in relation to the development of post-HSCT renal impairment. On multivariate analysis, only BK viremia (P = .000002) and alternative-donor transplantation (P = .002) were independent predictors of development of post-HSCT renal impairment, with BK viremia associated with a median 1.62 mg/dL rise in creatinine from the pretransplant baseline. Among 8 patients in the surveillance cohort with BK viremia, 2 developed biopsy-proven BKV nephropathy requiring hemodialysis. Investigation of whether prophylaxis against, or treatment of, BKV in the post-HSCT setting mitigates the associated morbidities, especially kidney injury, warrants prospective evaluation.


KEY WORDS: Allogeneic stem cell transplant, BK virus, Nephropathy

INTRODUCTION

BK virus (BKV), a human polyomavirus [1], was first recognized in 1971, after it was isolated from the urine of a Sudanese renal transplant patient who was hospitalized with acute renal failure and ureteral stenosis [2]. Most people are exposed to BKV during childhood, and 75% to 80% of adults have antibodies [3].

Primary infection occurs at 4 to 5 years of age, and manifests as a subclinical or nonspecific “flu-like” illness [4,5]. BKV then establishes latency primarily in the genitourinary tract [4,6], and viral reactivation generally occurs in immunocompromised patients [1,4]. BKV is an important pathogen and cause of nephropathy in recipients of renal transplants. Kidney transplant patients can become infected through reactivation of latent virus, through primary infection transmitted from the donor organ, or via blood transfusion [3]. Importantly, BKV infection can transition and escalate from viruria to viremia to nephropathy [7]. BKV nephropathy (BKN) begins as a localized viral presence in the tubular epithelial cells of the kidney, and progresses to a diffuse and destructive T cell-mediated interstitial nephritis [8]. The interstitial infiltrate can cause scarring of the kidney parenchyma, loss of function, and kidney failure. Since 1995, an increase in BKN from 1% in 1995 to 5% in 2001 has been observed in renal transplant patients [9].
In patients receiving hematopoietic stem cell transplantation (HSCT), the presence of BK viruria has long been appreciated [10], and BKV has been implicated in the pathogenesis of posttransplant hemorrhagic cystitis (HC) [10,11], although its exact role in that situation remains unclear [12]. HC with documented BK viruria is commonly treated with i.v. cidofovir, a drug with considerable nephrotoxic and myelosuppressive side effects [13]. Therefore, a better understanding of the clinical importance of BK viruria in the post-HSCT setting is needed. Furthermore, although BKN has been documented in native kidneys [5] and has recently been described in stem cell transplant patients [14,15], the prevalence and clinical importance of BK viremia and BKN in HSCT patients have not been previously described. We hypothesized that BKV infection is an important and underappreciated pathologic phenomenon associated with hematuria and the development of BKN in the post-HSCT setting. For a period of 16 months, we systematically monitored BKV infection in all allogeneic HSCT recipients to better understand the potentially important relationships between BK viruria, BK viremia, hematuria, and BKN in these patients.

MATERIALS AND METHODS

Study Participants

Beginning in September 2006, our transplant program implemented routine monitoring of BKV in the urine and blood of all adult patients who had already undergone, or who were undergoing, an allogeneic HSCT at the University of Chicago hospitals. All living transplant patients who presented for medical care, either as inpatients or outpatients, anytime after day 0 (their date of transplant), were monitored. Patients were enrolled into the study when their first BKV level (either urine or blood) was drawn. Data collection ended on December 31, 2007. One hundred twenty-four patients were enrolled. Sixty-seven had undergone transplant between September 2006 and December 2007, and were monitored from the time of transplant (the surveillance cohort). The data were analyzed in the spring of 2008. Institutional review board approval was received for analysis of the data.

Monitoring Methods and Data Included for Analysis

At any presentation to the University of Chicago Transplant Program for medical care during the period of study monitoring, HSCT patients submitted blood and urine samples for BKV detection and quantification. The frequency of sampling depended on how often the patient was seen. Typically, during a single inpatient stay, only 1 set of BKV samples (blood and urine) was collected, unless the patient had new hematuria later in the hospital stay. Blood and urine BKV samples were also collected at every outpatient visit. Patients were typically seen at least weekly until day 100 after transplant, at least every month until 6 months after transplant, at least 6 times between 6 months and 24 months after transplant, and at least once per year thereafter. The clinical data that were routinely captured from each inpatient or outpatient visit, when available, included: graft-versus-host disease (GVHD) assessment, microscopic urinalysis (U/A) results to measure urinary red blood cell (RBC) count, serum creatinine, blood tacrolimus level, and blood cytomegalovirus (CMV) status. Supportive care and infection prophylaxis at the University of Chicago transplant center have been previously described [16].

BKV Polymerase Chain Reaction (PCR) Analysis

Quantitative PCR was performed at the University of Chicago hospital laboratories on blood and urine samples to detect BKV DNA. Samples were placed in lysis buffer and stored at −70°C until processed. Using 200-μL aliquots of whole blood (in EDTA) or urine, DNA extraction was performed on the Roche MagNA Pure instrument (Roche Diagnostics, Indianapolis, IN), using the Roche Total NA kit, eluted into a final volume of 50 μL. In each extraction run, 2 different known concentrations of positive control containing target DNA were processed as well as a negative control containing bacterial DNA.

Master mix was prepared using the Roche FastStart Plus kit, and primers and probes were purchased from TIB MolBiol (Berlin, Germany). We used a multiplex assay for BK and JC viruses. The BKV target is the gene for large T antigen. To 5 μL of patient eluate was added 15 μL of master mix, and the samples were run on a Roche LightCycler 1.2. The protocol included 45 cycles of PCR. Five dilutions of a stock solution of cloned target DNA were used to generate a standard curve to determine the absolute quantification of DNA present in positive samples. Based on the standard curve, quantification was reported in a range from 2500 to 25,000,000 copies/mL of patient sample; positives outside the range are reported as >25,000,000 or <2500. The detection limit for the assay was validated as equivalent to 500 copies/mL of patient sample (10 copies/reaction). For positive samples, melting curve analysis was performed to verify the amplified product. An internal control was added to confirm negative samples.

Statistical Methods

The following variables were evaluated for their association with BK viruria and BK viremia: recipient sex, donor type, diagnosis, presence or absence of
GVHD, use of alemtuzumab in the conditioning regimen, presence of CMV viremia at any time during the monitoring period, and donor HLA C7 allele status. Donor HLA C7 allele status was included based upon evidence in renal transplant patients that absence of the HLA C7 allele in the donor is associated with development of BK viremia in the recipient [17].

To evaluate the potential effect of BK viruria or BK viremia on hematuria, hematuria was defined as absent, trivial (<3 RBC/high-power field [hpf] on urinalysis), or clinically significant (≥3 RBC/hpf). For each patient, correlation between hematuria and the median as well as the maximum level of BKV in the urine was investigated. The median level of BKV was the median of all samples obtained in an individual patient. The maximum concentration was the highest level measured in each individual patient. We also evaluated the correlation between presence of BK viremia at any time posttransplant and the occurrence of hematuria.

To evaluate the potential effect of BK viruria or viremia on kidney function, we measured the creatinine increment in each patient. This was defined as the difference between the serum creatinine at the time of transplant and the highest creatinine observed during the time of monitoring. We then evaluated the association between creatinine increment and the following factors: recipient sex, diagnosis, donor type, presence of acute or chronic GVHD, use of alemtuzumab in the conditioning regimen, presence of CMV viremia at any time during the monitoring period, presence of BK viruria at any time during the monitoring period, presence of BK viremia at any time during the monitoring period, presence of BK viremia at any time during the monitoring period, and donor HLA C7 allele status. Post-HSCT tacrolimus levels and their potential impact on creatinine changes were also considered in the analysis of each clinical variable’s effect.

Univariate statistical comparisons between patients based on the clinical factors of interest were analyzed using the Student t-test. All P values are 2-sided. The multivariate analysis of predictors of renal function decrement used multiple regression, with stepwise backward-elimination variable selection [18]. All covariates that were significant with a P value <.05 in the univariate analysis were retained in an initial regression model. (Because all patients with BK viremia also had BK viruria, and to determine whether BK viruria itself was an independent predictor, BK viruria in the absence of BK viremia was incorporated as its own covariate in the regression model, and BK viremia was incorporated as a separate covariate.) Factors not statistically significant (P ≥ .05) were removed from the model 1 at a time, with a reestimation of all model variables after each step. Variable elimination (or reinsertion) was stopped when all remaining factors were significant at P < .05.

Because of multiple comparisons in the multivariate analysis, we considered only P values <.01 as statistically significant.

### Table 1. Clinical and Transplant Characteristics of the 124 Patients and of the Prospective Cohort

<table>
<thead>
<tr>
<th>Patient Demographics</th>
<th>Entire Study Group</th>
<th>Prospective Cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>124</td>
<td>57</td>
</tr>
<tr>
<td>Median age (range)</td>
<td>49 (17-72)</td>
<td>52 (18-71)</td>
</tr>
<tr>
<td>Male</td>
<td>76 (61.3%)</td>
<td>38 (66.7%)</td>
</tr>
<tr>
<td>Female</td>
<td>48 (38.7%)</td>
<td>19 (33.3%)</td>
</tr>
<tr>
<td>Year of transplant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1999-2002</td>
<td>14 (11.3%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>2003-August 2006</td>
<td>54 (43.5%)</td>
<td>4 (7.0%)</td>
</tr>
<tr>
<td>September 2006-December 2007</td>
<td>56 (45.2%)</td>
<td>53 (93.0%)</td>
</tr>
<tr>
<td>Transplant donor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLA-identical</td>
<td>70 (56.5%)</td>
<td>29 (50.9%)</td>
</tr>
<tr>
<td>Matched-related</td>
<td>66 (53.2%)</td>
<td>27 (47.4%)</td>
</tr>
<tr>
<td>Mismatched-related</td>
<td>54 (43.5%)</td>
<td>28 (49.1%)</td>
</tr>
<tr>
<td>Mismatched-unrelated</td>
<td>36 (20.0%)</td>
<td>18 (31.6%)</td>
</tr>
<tr>
<td>Cord</td>
<td>10 (8.0%)</td>
<td>6 (10.5%)</td>
</tr>
<tr>
<td>Primary disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CML</td>
<td>8 (6.5%)</td>
<td>1 (1.8%)</td>
</tr>
<tr>
<td>CLL</td>
<td>5 (4.0%)</td>
<td>2 (3.5%)</td>
</tr>
<tr>
<td>AML</td>
<td>50 (40.3%)</td>
<td>25 (43.8%)</td>
</tr>
<tr>
<td>ALL</td>
<td>8 (6.5%)</td>
<td>2 (3.5%)</td>
</tr>
<tr>
<td>MDS</td>
<td>9 (7.3%)</td>
<td>4 (7.0%)</td>
</tr>
<tr>
<td>Aplastic anemia</td>
<td>3 (2.4%)</td>
<td>1 (1.8%)</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>27 (21.8%)</td>
<td>16 (28.0%)</td>
</tr>
<tr>
<td>Hodgkin lymphoma</td>
<td>5 (4.0%)</td>
<td>3 (5.3%)</td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td>3 (2.4%)</td>
<td>1 (1.8%)</td>
</tr>
<tr>
<td>Amyloidosis</td>
<td>1 (0.8%)</td>
<td>1 (1.8%)</td>
</tr>
<tr>
<td>Waldenstrom’s</td>
<td>1 (0.8%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Myelofibrosis</td>
<td>1 (0.8%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Pure red cell aplasia</td>
<td>1 (0.8%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>PNH</td>
<td>1 (0.8%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Plasmacytoid dendritic cell tumor</td>
<td>1 (0.8%)</td>
<td>1 (1.8%)</td>
</tr>
<tr>
<td>Conditioning regimen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fludarabine/melphalan/alemtuz</td>
<td>62 (50.0%)</td>
<td>26 (45.6%)</td>
</tr>
<tr>
<td>Fludarabine/busulfan/alemtuz</td>
<td>21 (16.9%)</td>
<td>13 (22.8%)</td>
</tr>
<tr>
<td>TBI/etoposide</td>
<td>9 (7.2%)</td>
<td>2 (3.5%)</td>
</tr>
<tr>
<td>Clofarabine/melphalan/alemtuz</td>
<td>8 (6.5%)</td>
<td>6 (10.5%)</td>
</tr>
<tr>
<td>Fludarabine/melphalan/ATG</td>
<td>5 (4.0%)</td>
<td>2 (3.5%)</td>
</tr>
<tr>
<td>TBI/fludarabine/thiotepa/ATG</td>
<td>4 (3.2%)</td>
<td>3 (5.3%)</td>
</tr>
<tr>
<td>TBI/cyclophosphamide</td>
<td>4 (3.2%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Fludarabine/cyclophosphamide</td>
<td>3 (2.4%)</td>
<td>2 (3.5%)</td>
</tr>
<tr>
<td>Fludarabine/BEAM</td>
<td>1 (0.8%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Busulfan/cyclophosphamide</td>
<td>1 (0.8%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Melphalan</td>
<td>1 (0.8%)</td>
<td>1 (1.8%)</td>
</tr>
<tr>
<td>TBI/etoposide/alemtuz</td>
<td>1 (0.8%)</td>
<td>1 (1.8%)</td>
</tr>
<tr>
<td>TBI/fludarabine</td>
<td>1 (0.8%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Graft-versus-host disease</td>
<td>40 (32.0%)</td>
<td>14 (24.6%)</td>
</tr>
<tr>
<td>Acute graft-versus-host disease, grade II-IV</td>
<td>67 (54.0%)</td>
<td>23 (40.4%)</td>
</tr>
<tr>
<td>CMV viremia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMV viremia at any time posttransplant</td>
<td>26 (21.3%)</td>
<td>16 (28.6%)</td>
</tr>
</tbody>
</table>

CML indicates chronic myelogenous leukemia; CLL, chronic lymphocytic leukemia; AML, acute myelogenous leukemia; ALL, acute lymphocytic leukemia; MDS, myelodysplastic syndrome; PNH, paroxysmal nocturnal hemoglobinuria; TBI, total body irradiation; alemtuz, alemtuzumab; ATG, antithymocyte globulin; BEAM, carmustine, etoposide, cytarabine, and melphalan.

The clinical characteristics of the 57 patients enrolled from day 0 of their transplant (the prospective cohort) were not statistically different from those of the entire study group.
RESULTS

Patient Characteristics

The clinical characteristics of the entire study cohort as well as the prospective cohort are summarized in Table 1. The entire cohort included patients undergoing HSCT as early as 1998, although the large majority was transplanted after 2002. The median age of participants at the time of their transplant was 49 years, and 61.3% of patients were males. Many diagnoses were included, but the predominant underlying diseases were acute myelogenous leukemia (AML) in 40.3% and non-Hodgkin lymphoma in 21.8%. The majority of patients (n = 70, 56.5%) received cells from an HLA-identical donor, defined as a matched-related (n = 66) or syngeneic donor (n = 4); the remainder received alternative-donor transplants, defined as matched-unrelated (n = 36), mismatched-related (n = 2), mismatched-unrelated (n = 6), cord (n = 4), or haploidential/cord (n = 6) donors. Most patients (n = 90, 72.6%) received conditioning regimens that included alemtuzumab, which is routinely used at our transplant center. Some form of GVHD (aGVHD and/or cGVHD) was diagnosed in 67 patients (54.0%), with 40 patients (32.0%) manifesting aGVHD (grade II-IV). In our study, 61 patients had donors lacking the HLA C7 allele (whereas, in another 18/124 patients, the donor HLA C7 status was unknown; 1 additional patient received a haploidential/cord transplant in which only 1 of the donor sources lacked HLA C7). CMV viremia was detected in 26 of 122 patients (21.3%) during the monitoring period.

Prevalence and Timing of Onset of BKV Infection

In the entire cohort, the median time after HSCT to first virus sampling was 58 days (range: 1-3196 days), and the median time to last follow-up virus sampling was 454 days (range: 3-3554 days). Seventy-nine patients (64.8%) had BK viruria at some time during monitoring post-HSCT. The remainder (43/122 = 35.3%) never

Table 2. Measure of the Degree to Which BKV Infection Status Changes for Individual Patients over Time in the post-HSCT Period

<table>
<thead>
<tr>
<th>BK Viruria</th>
<th>n</th>
<th>Median BKV Level (copies/mL)</th>
<th>Median Duration of Observation (days)</th>
<th>Median Duration of Positivity (days)</th>
<th>Median No. of Samples Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Persistently positive</td>
<td>27</td>
<td>25,000,000 (13,500-25,000,000)</td>
<td>157 (16-532)</td>
<td>157 (16-532)</td>
<td>11 (2-44)</td>
</tr>
<tr>
<td>Variable</td>
<td>52</td>
<td>2,500 (0-25,000,000)</td>
<td>322 (1-441)</td>
<td>32 (1-411)</td>
<td>11 (1-47)</td>
</tr>
<tr>
<td>Persistently negative</td>
<td>43</td>
<td>0 (0-0)</td>
<td>188 (1-429)</td>
<td>0 (0-0)</td>
<td>5 (1-21)</td>
</tr>
<tr>
<td>BK viremia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Persistently positive</td>
<td>2</td>
<td>1,147,250 (228,000-2,066,500)</td>
<td>286 (194-378)</td>
<td>286 (194-378)</td>
<td>22 (10-35)</td>
</tr>
<tr>
<td>Variable</td>
<td>19</td>
<td>0 (0-133,000)</td>
<td>220 (60-427)</td>
<td>1 (1-42)</td>
<td>17 (5-37)</td>
</tr>
<tr>
<td>Persistently negative</td>
<td>103</td>
<td>0 (0-0)</td>
<td>213 (1-532)</td>
<td>0 (0-0)</td>
<td>9 (1-47)</td>
</tr>
</tbody>
</table>

BKV indicates BK virus; HSCT, hematopoietic stem cell transplantation. Approximately one-quarter of all patients were persistently positive for BKV in the urine, whereas most fluctuated between exhibiting low-level positive and negative urine samples with a usual duration of positivity of approximately 1 month. In contrast, when BK viremia occurred it was almost always at low levels and was transient or intermittent. Only 2 patients had persistent BK viremia for the duration of the observation period. Notes: persistently positive patients were those who had 2 or more samples collected, and all samples were positive. Variable patients produced both positive and negative samples during the observation period, or they produced one positive sample and then had no other samples drawn. Persistently negative patients never exhibited a positive sample. In patients who had more than one period of viral positivity, the longest period was used to calculate the cohort median. Two patients did not have urinary BKV measured post-HSCT.

FIGURE 1. Cumulative incidence and timing of development of BK viruria and viremia for the prospectively monitored cohort (n = 57).
had BKV detected in the urine during post-HSCT follow-up. Two patients did not have urinary BKV measured post-HSCT (both died shortly after transplant, precluding obtaining of samples; neither had oliguria/anuria nor renal failure). Twenty-one patients (16.9%) had BK viremia detected at some time during monitoring. The remainder (103/124 = 83.1%) never demonstrated BKV in the blood post-HSCT.

Approximately one-quarter of all patients were persistently positive for BKV in the urine, whereas most others fluctuated between exhibiting low-level positive and negative urine samples with a usual duration of positivity of approximately 1 month (Table 2). In contrast, when BK viremia occurred, it was almost always at low levels and was transient or intermittent. Only 2 patients had persistent BK viremia for the duration of the observation period.

In the prospective cohort, the median time to first virus sampling was 20 days (range: 1-34 days), and the median time to last virus sampling was 146 days (range: 3-454 days). Among these patients, 40 (72.7%) developed BK viruria and 13 (22.8%) developed BK viremia. In this group, the median time from transplantation to development of BK viruria was 24 days (range: 3-138 days), whereas BK viremia occurred at a significantly longer median time of 128 days (range: 62-307 days) \((P = .001)\). The cumulative incidence and timing of development of BK viruria and viremia for the prospective cohort are depicted in Figure 1.

### Clinical Factors Having an Impact on BKV Infection

Because previous studies have suggested that increased age is associated with an increased prevalence of BK viruria, at least in immunocompetent populations \[19,20\], we analyzed the median age of our patients who had BK viruria and compared it to the median age of our patients without urinary BKV. The median age of the 2 groups was not statistically different (BK viruria group = 48 years, no urinary BKV group = 52 years, \(P = .07\)).

In a univariate analysis of all of the clinical factors examined, only CMV viremia was significantly associated with an increased risk of also having BK viruria \(P = .001\) and BK viremia \(P = .04\) (Table 3). Notably, BK viruria and viremia were not more prevalent in patients receiving alemtuzumab.

### Relationship Between BKV Infection and Hematuria

One hundred twenty of the 124 study patients had at least 1 U/A performed. Only 19 patients (15.8%) never demonstrated microscopic hematuria at any time (“no hematuria group”). Most patients with microscopic hematuria had trivial hematuria \((n = 84/101)\). Seventeen patients had clinical hematuria.

The dichotomous presence or absence of BK viruria in the post-HSCT period was not a predictor of development of hematuria, because there was no difference in the prevalence of BK viruria in those who demonstrated hematuria compared to those who did not. There was, however, a significant association between the median amount of BK viruria and the degree of hematuria. The average of all patients’ median BK viruria levels was 1.99(10^6) copies/mL in the “no hematuria” group, lower than the average of the median BK viruria levels in the “trivial hematuria” group (5.44(10^5) copies/mL, \(P = .06\)), which in turn, was significantly lower than the average of the median BK viruria levels in the “clinical hematuria” group (13.32(10^6) copies/mL, \(P = .03\)) (Figure 2). This suggests that there is a direct relationship between the median amount of urinary BKV in the post-HSCT period and both the presence, and the degree, of microscopic hematuria.

### Table 3. Association between Various Clinical Factors and Prevalence of BK Viruria and Viremia

<table>
<thead>
<tr>
<th>Clinical Factor</th>
<th>BK Viruria</th>
<th>BK Viremia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td>0.48</td>
<td>0.06</td>
</tr>
<tr>
<td>Males</td>
<td>0.48</td>
<td>0.06</td>
</tr>
<tr>
<td>HLA identical donor</td>
<td>0.09</td>
<td>0.06</td>
</tr>
<tr>
<td>Alternative donor</td>
<td>0.77</td>
<td>0.53</td>
</tr>
<tr>
<td>CLL</td>
<td>0.53</td>
<td>0.53</td>
</tr>
<tr>
<td>AML</td>
<td>0.47</td>
<td>0.53</td>
</tr>
<tr>
<td>ALL</td>
<td>0.47</td>
<td>0.53</td>
</tr>
<tr>
<td>MDS</td>
<td>0.47</td>
<td>0.53</td>
</tr>
<tr>
<td>AA</td>
<td>0.47</td>
<td>0.53</td>
</tr>
<tr>
<td>NHL</td>
<td>0.47</td>
<td>0.53</td>
</tr>
<tr>
<td>HD</td>
<td>0.47</td>
<td>0.53</td>
</tr>
<tr>
<td>MM</td>
<td>0.47</td>
<td>0.53</td>
</tr>
<tr>
<td>Alemtuzumab</td>
<td>0.47</td>
<td>0.53</td>
</tr>
<tr>
<td>No alemtuzumab</td>
<td>0.47</td>
<td>0.53</td>
</tr>
<tr>
<td>GVHD</td>
<td>0.47</td>
<td>0.53</td>
</tr>
<tr>
<td>Donor HLA C7 positive*</td>
<td>0.47</td>
<td>0.53</td>
</tr>
<tr>
<td>Donor HLA C7 negative*</td>
<td>0.47</td>
<td>0.53</td>
</tr>
<tr>
<td>CMV viremia†</td>
<td>0.47</td>
<td>0.53</td>
</tr>
<tr>
<td>No CMV viremia†</td>
<td>0.47</td>
<td>0.53</td>
</tr>
</tbody>
</table>

CML indicates chronic myelogenous leukemia; CLL, chronic lymphocytic leukemia; AML, acute myelogenous leukemia; ALL, acute lymphocytic leukemia; MDS, myelodysplastic syndrome; AA, aplastic anemia; NHL, non-Hodgkin lymphoma; HD, Hodgkin disease; MM, multiple myeloma. NT, not tested; GVHD, graft-versus-host disease.

*For donor HLA C7 allele status, information was not available for 18 patients, and 1 patient received a haploidentical/cord transplant in which only 1 of the donor sources was positive for the HLA C7 allele—this patient was not included in the above table.

†For the presence of CMV viremia, 2 patients did not have blood CMV samples drawn.
The group with no hematuria had an average maximum urinary BKV level of 5.27(10^6) copies/mL, which was not significantly different than that of those with trivial hematuria (8.44(10^6) copies/mL, P = .25). Yet, both groups’ levels were significantly lower than the maximum BK viruria levels of those with clinical hematuria (17.65(10^6) copies/mL, P < .007) (Figure 2). These data suggest that maximum BKV urinary load, even as measured by 1 U/A, is directly associated with both the occurrence, and the degree, of hematuria.

We also examined whether BK viremia was associated with hematuria. In our study, interestingly, all 19 patients without hematuria were BK blood negative, whereas 21 of 101 patients with hematuria were BK blood positive (P = .03).

Impact of BKV Infection on Renal Function

Nearly all transplant patients were observed to incur a decrement in renal function simply by undergoing HSCT. The median baseline (day 0) serum creatinine value for the entire cohort was 0.80 mg/dL. This was significantly lower than the median of the peak post-HSCT creatinine values for the cohort, 1.30 mg/dL (P < 10^-12). Of the 124 patients, only 12 did not have higher maximal post-HSCT creatinine values compared to their day 0 creatinine baselines.

On univariate analysis, 6 clinical factors were associated with a concomitant decrement in renal function: female sex, having undergone an alternative-donor transplant, having an underlying disease that was not myelodysplastic syndrome (MDS), having CMV viremia, having BK viruria, and having BK viremia (Table 4). Median post-HSCT tacrolimus levels for patients with these characteristics were not significantly higher and did not explain any of these associations. On multivariate analysis, only 2 factors were independently associated with a significant change in creatinine: undergoing an alternative-donor transplant (P = .002) and having BK viremia (P = .000002). The factor with the greatest absolute effect on creatinine elevation was BK viremia: the creatinine rise was 1.62 mg/dL in patients with BK viremia, but only 0.64 mg/dL in those without BKV in the blood (P = .001) (Figure 3).

Development of BKV Nephropathy

Among the 8 patients in the surveillance cohort with BK viremia, 2 developed biopsy-proven BKV-associated interstitial nephritis with severe renal failure. No other cases of unexplained renal failure requiring kidney biopsy occurred. Interestingly, the 2 patients with BKV nephropathy were the only 2 to have persistently positive blood BKV levels throughout the period of monitoring (Table 2). A description of these 2 cases of BKV nephropathy is provided here.

The first patient was a 36-year-old female with relapsed Hodgkin disease (HD) who had previously undergone an autologous HSCT 1 year prior. Her conditioning regimen prior to matched, unrelated donor transplantation consisted of fludarabine (Flu), melphalan (Mel), and alemtuzumab. She had no prior history of kidney disease. Transplant was complicated with steroid-dependent GVHD. Her renal function began to slowly decline after transplant. BK viremia was detected, and without another cause of her renal insufficiency identified, a kidney biopsy was performed 9 months after transplant. The biopsy revealed changes reflective of polyomavirus nephropathy: a diffuse, prominent, interstitial inflammatory reaction with tubulitis (Figure 4A). These cells tested strongly positive using an SV40 immunohistochemical stain (SV40 and BKV share a significant region of homology) [21] (Figure 4B). Urinary BKV levels were consistently at the upper limit of the assay’s detection throughout this period, whereas blood BKV levels were modestly and consistently elevated.
The patient was treated with leflunomide, an immunomodulatory agent, but there was no appreciable decrease in her blood or urine BKV levels, and her glomerular filtration rate continued to worsen. She required initiation of hemodialysis 18 months after her transplant.

The second patient was a 41-year-old female with AML who underwent matched-unrelated donor HSCT in first remission after Flu/Mel/alemtuzumab conditioning. Two and a half years after her transplant, she began to develop acute renal insufficiency, which corresponded temporally to a rapid spike in her blood BKV measurements to very high levels. Urinary BKV levels were also consistently at the upper limit of the assay’s detection. An alternative cause of her acute renal insufficiency was not found. Leflunomide was given, without a dramatic decrease in blood or urine BKV levels. Kidney biopsy revealed polyomavirus ne-

Table 4. Average Creatinine Rise Post-HSCT as a Function of Various Clinical Factors

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Creatinine Rise of</th>
<th>Comparison Group (mg/dL)</th>
<th>Difference</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Creatinine Value</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female sex</td>
<td>48 1.02 0.67</td>
<td>0.35 .04</td>
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<td></td>
</tr>
<tr>
<td>Alternative-donor</td>
<td>54 1.10 0.58</td>
<td>0.52 .0009</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CML</td>
<td>8 0.71 0.81</td>
<td>-0.10 .67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CLL</td>
<td>5 0.88 0.80</td>
<td>0.08 .60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AML</td>
<td>50 0.96 0.70</td>
<td>0.26 .12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALL</td>
<td>8 1.01 0.79</td>
<td>0.22 .41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDS</td>
<td>9 0.47 0.83</td>
<td>-0.36 .02</td>
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<td></td>
</tr>
<tr>
<td>NHL</td>
<td>27 0.60 0.86</td>
<td>-0.26 .07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HD</td>
<td>5 1.42 0.78</td>
<td>0.64 .46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematuria</td>
<td>101 0.87 0.62</td>
<td>0.25 .26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alemtuzumab</td>
<td>90 0.81 0.78</td>
<td>0.03 .93</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GVHD</td>
<td>68 0.90 0.69</td>
<td>0.21 .17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Donor HLA C7 positive</td>
<td>45 0.93 0.78</td>
<td>0.15 .41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMV viremia</td>
<td>26 1.21 0.70</td>
<td>0.51 .03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BK viremia</td>
<td>79 0.97 0.53</td>
<td>0.44 .001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BK viremia</td>
<td>21 1.62 0.64</td>
<td>0.98 .001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CML indicates chronic myelogenous leukemia; CLL, chronic lymphocytic leukemia; AML, acute myelogenous leukemia; ALL, acute lymphocytic leukemia; MDS, myelodysplastic syndrome; NHL, non-Hodgkin lymphoma; HD, Hodgkin disease; MM, multiple myeloma; GVHD, graft-versus-host disease; CMV, cytomegalovirus.

For each clinical factor listed, the comparison group is comprised by all patients not demonstrating that clinical characteristic (eg, for “females,” the comparison group is all males; for “HLA alternative” the comparison group is all patients who received an HLA-identical transplant; for “BK viremia” the comparison group is all patients without BK viremia; for the disease subtypes, the comparison group is all other patients in the study who did not have that disease). Patients with aplastic anemia (n = 3), myeloma (n = 3), and 1 of the 6 other singularly represented diseases (n = 6) were not tested because of the small sizes of these subcategories. On univariate analysis, 6 of the above clinical factors were significantly associated with a concomitant decrement in renal function: female sex; having undergone an alternative-donor transplant; having an underlying disease that was not MDS; having CMV viremia; having BK viruria; and having BK viremia. Patients with MDS had a significantly smaller rise in creatinine (0.47 mg/dL) than all other patients (0.83 mg/dL, P = .02). On multivariate analysis, only 2 factors were independently associated with a significant change in creatinine: undergoing an alternative-donor transplant (P = .002) and having BK viremia (P = .000002).

The result of this analysis of a large, systematically monitored cohort of patients demonstrates that BK viruria is a common finding among HSCT recipients, whereas BK viremia is less common, but not rare. BK viruria is detectable early after HSCT, and, in the cohort of patients who were tracked from day 0, the timing of detection of BK viruria (24 days) was similar to the median timing of first virus sampling (20 days), suggesting that BK viruria may actually be detectable even earlier, and, in a portion of patients, is likely present at baseline (pretransplant). Although we did not measure this, BKV shedding can be detected in the urine of a portion of immunocompetent individuals [22,23] and, in a recent preliminary analysis, was detected in 46% of transplant patients pretransplant [24]. The prevalence of post-HSCT BK viruria in our study (64.8%) is similar to multiple other studies that have demonstrated rates between 50% and 100% [25-29].

In contrast, BK viremia has been less well-studied, and its timing of detection in our post-HSCT population was significantly later than that of BK viruria, suggesting that BK viremia represents a separate, pathologic disease state in this patient population. Several hypotheses have been purported [3] in kidney transplant recipients, which may explain the late occurrence of BK viremia in our patients: BKV may be acquired via blood transfusions (a risk that would increase over time in patients because of the chronologic cumulative need for transfusions post-HSCT); it could represent acquisition of BKV from the donor, with a period of latency or required replication post-HSCT until detection is possible; or it may represent a form of native “progression” of BKV infection in the transplant recipient, possibly in a step-wise manner (genitourinary BKV latency → detectable BK viruria → frank BK viremia) [7,9,14,30,31]. Our study did not measure BKV in the blood of donors, nor does our hospital routinely screen for BKV in transfused blood products, so we could not definitively differentiate between these possibilities in our study. However, the fact that all patients who had BK viremia also had BK viruria, and the fact that BK viruria is detectable much earlier, lends credence to the “progression” hypothesis.

Analysis of several transplant-related clinical factors potentially associated with the development of BKV infection was undertaken in this study, but none of the common predictors of transplant-related disease had a significantly associated with a concomitant decrement in renal function: undergoing an alternative-donor transplant; having an underlying disease that was not MDS; having CMV viremia; having BK viruria; and having BK viremia. Patients with MDS had a significantly smaller rise in creatinine (0.47 mg/dL) than all other patients (0.83 mg/dL, P = .02). On multivariate analysis, only 2 factors were independently associated with a significant change in creatinine: undergoing an alternative-donor transplant (P = .002) and having BK viremia (P = .000002).

The patient was treated with leflunomide, an immunomodulatory agent, but there was no appreciable decrease in her blood or urine BKV levels, and her glomerular filtration rate continued to worsen. She required initiation of hemodialysis 18 months after her transplant.
outcomes was associated with an increased (or decreased) occurrence of BK viruria or viremia. Having GVHD and undergoing an HLA-mismatched transplant had specifically been previously implicated as possible risk factors for BKV reactivation [25,32], but these associations were not found in our study. Only CMV viremia was found to be associated with higher rates of BK viruria and viremia. One prior case report also suggested a potential correlation between CMV and BKV reactivation [33]. Although causation cannot be determined by our data, we do not believe that CMV viremia predisposes a patient to the development of BKV infection. Rather, it is more likely that the as-yet-poorly defined factors that control CMV reactivation/replication in patients in the post-HSCT period are the same factors that govern this process for BKV.

Our data add supporting evidence to prior studies that have implicated urinary BKV in the development of post-HSCT hematuria [11]. Our findings that both a higher continuous level of urinary BKV and a higher maximum urinary BKV level are associated with greater numbers of RBC in the urine are consistent with prior similar associations [28,29,34,35]. Although we did not assess the clinical severity of episodes of hematuria (i.e., whether there were associated urinary symptoms), our data do support the hypothesis that higher levels of urinary BKV may be correlated with greater urinary bleeding. Our finding of an association between BK viremia and hematuria was consistent with findings from 2 prior studies [36,37], but in contrast to another report that found no correlation [28]. Given that hematuria (and HC) are problematic and potentially serious HSCT complications [38], our data would support prospective testing of the concept that pharmacologic reduction in urinary BKV load, if achievable, might prevent more severe cases of urinary bleeding as can be seen with severe HC.

The most intriguing conclusion from this study was the demonstration of BK viremia as an independent risk factor for deterioration of renal function in the post-HSCT setting. Previously, only case reports had described an association between BKV and renal failure post-HSCT [14,15,39,40]. Although HSCT itself resulted in an aggregate rise in creatinine above pretransplant baselines in our study, undergoing an alternative-donor transplant and having BK viremia emerged as being independently associated with a statistically significant increase beyond the increase seen with transplantation alone. The absolute increase in creatinine observed in patients with BK viremia (median 1.62 mg/dL) was large, and had the strongest statistical association. Even in the absence of frank oliguric renal failure, this degree of rise in serum creatinine represents a significant decrement in glomerular filtration rate, which can complicate the ongoing treatment of patients especially as it has an impact on clearance of many transplant-related medications, precludes contrast-related radiologic imaging, and complicates handling of fluid volumes accompanying intravenous medications and blood products.

In contrast to BK viruria, which often persisted during the period of observation, BK viremia was transient in many cases and so were elevations in creatinine. Still, 2 of 8 patients in the surveillance cohort (which represents the patients with prolonged follow-up) developed severe interstitial nephritis that was biopsy-proven as BKV
mediated. Given that these 2 individuals were the only 2 to have persistently positive blood BKV levels in our study, this may suggest that prolonged duration of BK viremia is a risk factor for developing BKV nephropathy. It is also likely that at least some of the other patients in our study with impairment of renal function had undiagnosed mild BKV nephropathy [14,15]. Thrombotic thrombocytopenic purpura (TTP)-associated renal failure was not a confounder of the BK viremia-renal impairment association, as only 2 patients developed TTP in our study and neither had BK viremia. In a portion of the cases with BK viremia (10 of 21, including the 2 patients with biopsy-proven BKV nephropathy and renal failure), the occurrence of renal impairment temporally followed the patient turning positive for blood BKV or a spike in blood BKV levels, suggesting a direct causative relationship. In the other BK viremic patients, no clear temporal relationship between the timing of viremia and creatinine rise was observed, and the inciting event causing acute renal impairment was likely another cause. In these cases where BK viremia itself was not directly causative of renal impairment, it is possible that the predisposing factors for renal impairment—perhaps in the local microenvironment of the kidney itself—are the same, which predispose to BKV reactivation and BK viremia, thereby explaining the strong association that was found.

One limitation of this study is that we were not able to account for all differences that may have existed in the administration of all potentially nephrotoxic concomitant medications post-HSCT, although we did control for tacrolimus, perhaps the most commonly used nephrotoxic agent in our patients. Although almost all patients at our center are treated with the same prophylactic medical regimens post-HSCT, patient-specific differences in the use of other chronic medications were not accounted for in our analysis. The use of aminoglycosides is specifically avoided in our transplant population in usual practice, and aminoglycosides were not administered to either of the 2 patients who ultimately developed renal failure requiring hemodialysis. In considering the impact of conditioning regimens on our outcomes, use of total body irradiation (TBI) and/or cyclophosphamide (Cy) did not have an impact on creatinine change. We also evaluated whether the use of alemtuzumab, a potent immunosuppressant [41,42], which is favored at our center, had an impact on the incidence or severity of BK viruria or viremia. Importantly, BKV infection was not increased in patients receiving alemtuzumab. Separately, we cannot rule out the possibility that some patients had more frequent virus sampling because of unmeasured variables, such as increased posttransplant complications. Finally, this study was conducted at 1 urban transplant center, and transplant-related supportive care practices that were not captured in our analysis are not necessarily similarly implemented at other centers.

In summary, BKV infection is common in post-HSCT patients, is associated with hematuria, and

Figure 4. Photomicrographs of the pathologic findings in 2 patients with biopsy-proven BK virus-induced nephropathy. (A) (top panel) High-powered view of enlarged tubular epithelial cells showing viral cytopathic effect and intranuclear inclusions in 1 of the 2 affected patients in our study. Glomeruli and vascular structures are normal; (B) (middle panel) SV40 immunohistochemical stain further demonstrating the intranuclear viral inclusions in the same patient (SV40 and BKV share a significant region of homology); (C) (bottom panel) similar pathologic findings in the kidney of the second patient in our study with BKV nephropathy.
BK viremia, possibly by causing kidney damage that can lead to BKV nephropathy in some cases, is an independent risk factor for worsening renal function in the post-HSCT period. Investigation of whether prophylaxis against, or treatment of, BKV in the post-HSCT setting mitigates the associated morbidities, especially kidney injury, is the subject of ongoing investigation.

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Contributions


REFERENCES

Supplemental Figure S1. Relationship between timing and magnitude of BK viremia and creatinine rise in the 2 patients who had persistently positive blood BKV levels and who developed BKV nephropathy and end stage renal failure. (A) (top panel) corresponds to the first patient described in the text; (B) (bottom panel) corresponds to the second patient described in the text.