Polyomavirus JC Urinary Shedding in Kidney and Liver Transplant Recipients Associated With Reduced Creatinine Clearance

Shimon Kusne,1 Regis A. Vilchez,3,a Preeti Zanwar,3 Jorge Quiroz,4 Marek J. Mazur,1 Raymond L. Heilman,1 David Mulligan,2 and Janet S. Butel3

Departments of 1Medicine, 2Transplant Surgery, Mayo Clinic Arizona, Scottsdale, Arizona; 3Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, Texas; and 4Department of Biostatistics, Merck Research Laboratories, Kenilworth, New Jersey

Background. Polyomavirus reactivation can cause significant morbidity in solid organ transplant recipients, particularly BK virus (BKV) in kidney transplant patients. Less is known about dynamics of John Cunningham virus (JCV) in nonkidney organ transplant patients.

Methods. We examined the frequency of urinary shedding of polyomaviruses BKV and JCV and their relationship to creatinine clearance (CrCl) in a longitudinal study of 41 kidney and 33 liver transplant recipients.

Results. Any polyomavirus urinary shedding was more frequent in liver than kidney recipients (64% vs 39%; \( P = .03 \)). JCV was excreted more frequently by liver than kidney recipients (71% vs 38%), whereas BKV was shed more often by kidney than liver patients (69% vs 52%). Mean JCV loads were significantly higher than those of BKV in both patient groups (\( P < .0001 \)). Lower mean CrCl values were significantly associated with JCV shedding in both kidney and liver recipients (\( P < .001 \)).

Conclusions. These findings suggest that BKV and JCV display different patterns of reactivation and shedding in kidney and liver transplant patients and that JCV may have a role in renal dysfunction in some solid organ transplant recipients.

BK virus (BKV) and John Cunningham virus (JCV) are nonenveloped icosahedral DNA viruses, members of the family Polyomaviridae. Studies have estimated that the adult population worldwide is approximately 80% seropositive for BKV and approximately 50%–70% seropositive for JCV, with JCV seropositivity increasing with age [1–3]. Primary infection normally occurs during childhood, with the viruses then establishing latency/persistence in different organs, including the kidney [4, 5]. BKV and JCV undergo periodic reactivation and replication, and may cause disease in immunosuppressed hosts [6–10]. It is not known exactly which factors control the balance between latency and reactivation of BKV and JCV, but available data suggest that the cellular immune response exerts important control over these viruses [6, 11–14]

BKV is known to cause diseases of the genitourinary tract, such as hemorrhagic cystitis in bone marrow and hematopoietic stem cell transplant recipients and ureteric stenosis in renal transplant patients. However, the virus is most frequently implicated with the development of polyomavirus-associated nephropathy (PVAN) in kidney transplant patients [6, 9, 15, 16]. Reduced host immunity seems to play an important role, as studies indicate that lowering of the level of immunosuppression is associated with a decrease in BKV viral load and reduction of allograft inflammation in kidney transplant patients [6, 8, 15, 16]. Progressive multifocal leukoencephalopathy is a disease of the central nervous system, characterized by multiple
foci of demyelination caused by lytic JCV infection of oligodendrocytes [7, 10, 17, 18]. Progressive multifocal leukoencephalopathy has been reported among heart, kidney, and liver transplant recipients, but its true incidence in these patient groups is not known [6, 19–21]. In addition, JCV has also been associated with some cases of PVAN in kidney transplant recipients [22–24]. Data suggest that JCV-associated PVAN may be characterized by sparse cytopathic changes but significant inflammation and fibrosis in kidney transplant patients [24]. However, the relationship between JCV reactivation and renal dysfunction is not clear, as systematic monitoring of JCV infection is not performed in kidney and nonkidney transplant patients.

A high incidence of renal dysfunction has been reported in nonrenal transplant recipients [25, 26]. This renal disease has been attributed to the cumulative toxicity of calcineurin inhibitors, but many of these patients are not monitored for polyomavirus reactivation, so it is possible that polyomaviruses are more commonly associated with this clinical syndrome than currently appreciated. There is a need, therefore, for prospective studies to examine the role of BKV and JCV in renal dysfunction among nonrenal organ transplant patients. In addition, some questions remain concerning the clinical management of BKV and JCV following organ transplantation, such as the dynamics of reactivation of individual viruses in different organ transplant groups and the advisability of viral monitoring. In this prospective study, we examined BKV and JCV urinary shedding and their relationship with creatinine clearance (CrCl) in outpatient liver and kidney transplant recipients to determine whether the patterns of viral reactivation were similar in the 2 patient groups and if viral shedding was associated with renal dysfunction in liver transplant recipients.

**PATIENTS AND METHODS**

**Study Population**

Adult kidney and liver transplant recipients who had received a transplant operation and medical care at Mayo Clinic, Arizona, were enrolled and monitored prospectively from January 2005 through May 2007. Patients were eligible if they were receiving immunosuppressive agents and were ambulatory. Immunosuppressive agents are used to prevent rejection as induction immediately after the transplant operation and as maintenance therapy or as treatment of acute and chronic rejection. The mechanisms of action of these agents have been described [27, 28]. Patients were excluded if they were undergoing dialysis. All patients signed informed consent. The study was approved by the Mayo Clinic (protocol 109-04) and Baylor College of Medicine (protocol H-17200) institutional review boards.

Standard demographic and historical data were collected on each patient. At each visit, clinical information regarding serum creatinine, body weight, and current immunosuppressive regimen were collected. CrCl rates were calculated at each clinic visit with a standard Cockcroft–Gault formula using the corresponding serum creatinine and patient body weight [29].

**Sample Collection and Virological Analysis**

Urine and blood samples were collected from patients at approximately 3-month intervals after enrollment at the time of routine clinic visits. Heparinized blood samples were placed upright for 2 hours, then the plasma and approximately 400 μL of cells at the interface were pipetted off. This leukocyte-rich plasma was centrifuged at 1800 rpm for 6 minutes, the plasma was removed, and the cell pellet of peripheral blood leukocytes (PBLs) was resuspended in 1 mL phosphate-buffered saline. Both cells and plasma were frozen at −70°C. Urine samples were obtained in sterile collection cups and spun at low speed (2000g) for 10 minutes at room temperature to pellet cellular material, and the supernatants were separated from the pellets and stored at −70°C. Frozen samples were shipped to the Department of Molecular Virology and Microbiology, Baylor College of Medicine, for blinded laboratory analysis. Sample processing and DNA extractions were performed in a laminar flow hood within a BSL2+ facility free from viruses and plasmids following procedures reported previously [30, 31].

DNA was extracted from PBL and urine samples using the Gentra Puregene Tissue Kit (Qiagen, Valencia, CA), following the manufacturer’s instructions. DNA samples were tested by conventional polymerase chain reaction (PCR) using assay conditions and universal polyomavirus primers able to detect JCV and BKV sequences. Each reaction contained 0.5–1μg PBL DNA (approximately 8–16 × 10⁴ cell equivalents) or DNA extracted from 1.5–2.0 mL urine [30, 31]. These assays had a limit of detection of 100 genome copies/reaction [32]. Samples that were positive by conventional PCR were then assayed by real-time quantitative PCR (RQ-PCR) to identify the specific virus present (BKV or JCV) and to determine viral loads. Specific primers and probes directed against the N-termini of the large T-antigen genes of BKV and JCV and assay conditions and performance characteristics have been described [32]. The limit of detection of these RQ-PCR assays was 10 genome copies/reaction.

**Data Analysis**

A logistic regression model was used to examine whether the proportion of patients shedding polyomaviruses was different between kidney and liver transplant recipients. An analysis of variance (ANOVA) model was used to compare the viral load of kidney and liver transplant patients shedding either BKV or JCV. Multiple viral loads were obtained on patients that visited the transplant center more than once. Therefore, since measurements on the same subject were correlated, it was
necessary to accommodate for the correlation present in the data (ie, subject variability was incorporated in the ANOVA model). The statistical analysis was conducted in logarithm base 10 (log10) scales to reduce the skewness of the polyomavirus viral loads and to improve the variance estimation.

An ANOVA model was also used to compare the CrCl value, depending on whether patients were shedding BKV or JCV or no virus and whether patients were kidney or liver transplant recipients. The ANOVA model used to compare CrCl also accommodated for the subject correlation present in the data.

There were no censored values for polyomavirus viral loads or CrCl values. Statistical differences were declared based on the traditional statistical significance level of 5%. All the statistical analyses were performed using the statistical software SAS version 9.1.

RESULTS

Seventy-four outpatient organ transplant patients (41 kidney and 33 liver recipients) were enrolled and monitored between January 2005 and May 2007; this represented 785 patient-years of follow-up. The demographic and clinical characteristics of the patients are presented in Table 1. The mean time between transplantation and enrollment into the study was 1.47 years (range, 13 days to 9 years). Immunosuppressive agents included tacrolimus (n = 71), cyclosporine A (n = 44), mycophenolate mofetil (n = 62), and sirolimus (n = 1). The majority of patients in both groups received tacrolimus (95% and 97%, respectively), cyclosporin was used mainly in kidney transplant recipients (100% vs 9%), and mycophenolate treatment was used more frequently among kidney than liver transplant patients (98% and 67%, respectively). The mean number of urine samples obtained per patient during the course of the study was 3.4 (range, 1–8); a total of 251 urine samples were analyzed. There were 6 deaths (2 kidney and 4 liver recipients) during the study period, none of which was related to polyomaviruses.

Detection of Polyomavirus BKV and JCV DNA in Urine and Blood Samples

The frequency of polyomavirus urinary shedding by kidney and liver transplant recipients is presented in Table 2. Overall, 50% (37/74) of the patients were found to shed polyomavirus in the urine. Among those positive virus shedders, 70% (n = 26) were excreting polyomavirus at multiple clinic visits. The proportion of virus-shedding liver transplant patients was greater than shedders among the kidney transplant recipients (21/33, 64% vs 16/41, 39%; P = .03).

The frequency of BKV and JCV shedding displayed different patterns among the 2 transplant groups (Table 2). The proportion of patients shedding BKV was higher in kidney than in liver transplant recipients (69% vs 52%). In contrast, the proportion of patients shedding JCV was higher in the liver than the kidney transplant group (71% vs 38%). There was 1 kidney transplant recipient and 5 liver transplant patients who shed both polyomaviruses concurrently. These were single occurrences, except for 1 liver transplant patient who had 2 doubly positive urine samples collected 8 months apart. Three of the kidney transplant patients shedding BKV in the urine also had detectable levels of virus in their PBL samples. One patient was positive for BKV viremia in 2 blood samples collected 1 year apart. None of the patients with JCV in the urine had virus detected in the blood.

Quantification of Polyomavirus BKV and JCV Viral Loads in Urine Samples

The overall quantitative mean viral loads (log10) in urine samples were compared for organ transplant group (kidney vs liver) and for polyomavirus type (BKV vs JCV) (Table 3). The mean urinary polyomavirus load was higher in kidney than in liver transplant patients (log10 6.20 copies/mL vs log10 5.64 copies/mL).

Table 1. Demographic and Immunosuppressive Drugs of Kidney and Liver Transplant Recipients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Kidney Transplant Recipients (n = 41)</th>
<th>Liver Transplant Recipients (n = 33)</th>
<th>Total (n = 74)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female</td>
<td>19/22</td>
<td>28/5</td>
<td>47/27</td>
</tr>
<tr>
<td>Immunosuppressive agents</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>39 (95%)</td>
<td>32 (97%)</td>
<td>71 (96%)</td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>41 (100%)</td>
<td>3 (9%)</td>
<td>44 (59%)</td>
</tr>
<tr>
<td>Mycophenolate mofetil</td>
<td>40 (98%)</td>
<td>22 (67%)</td>
<td>62 (84%)</td>
</tr>
<tr>
<td>Sirolimus</td>
<td>0 (0%)</td>
<td>1 (3%)</td>
<td>1 (1%)</td>
</tr>
</tbody>
</table>

Table 2. Frequency of BKV and JCV Urinary Shedding by Kidney and Liver Transplant Recipients

<table>
<thead>
<tr>
<th>Virus detected</th>
<th>Kidney Transplant Recipient (%)</th>
<th>Liver Transplant Recipient (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No virus</td>
<td>25/41 (61)</td>
<td>12/33 (36)</td>
<td>37/74 (50)</td>
</tr>
<tr>
<td>Any polyomavirus</td>
<td>16/41 (39)</td>
<td>21/33 (64)</td>
<td>37/74 (50)</td>
</tr>
<tr>
<td>BKV only</td>
<td>10/16 (63)</td>
<td>6/21 (28)</td>
<td>16/37 (43)</td>
</tr>
<tr>
<td>JCV only</td>
<td>5/16 (31)</td>
<td>10/21 (48)</td>
<td>15/37 (40)</td>
</tr>
<tr>
<td>Both BKV and JCV</td>
<td>1/16 (6)</td>
<td>5/21 (24)</td>
<td>6/37 (16)</td>
</tr>
<tr>
<td>Total BKV</td>
<td>11/16 (69)</td>
<td>11/21 (52)</td>
<td>22/37 (59)</td>
</tr>
<tr>
<td>Total JCV</td>
<td>6/16 (38)</td>
<td>15/21 (71)</td>
<td>21/37 (57)</td>
</tr>
</tbody>
</table>

Abbreviations: BKV, BK virus; JCV, John Cunningham virus.
* The sum of numbers for total BKV and total JCV is greater than patient numbers in a given transplant cohort because dual virus shedders are included in both virus groups.
copies/mL; \( P = .043 \). The mean JCV viral loads were significantly higher than those of BKV in both transplant groups (log10 6.64 copies/mL vs log10 5.20 copies/mL; \( P < .0001 \)).

**CrCl and Polyomavirus BKV and JCV Urinary Shedding**

The relationships of polyomavirus shedding to mean and median CrCl values in kidney and liver transplant recipients are shown (Figure 1). The lowest mean CrCl in the kidney transplant group was observed in recipients who were shedding JCV (55.86 mL/min) compared to BKV (69.77 mL/min) and no polyomavirus shedders (57.43 mL/min; \( P < .001 \)). A similar finding was observed in the liver transplant group; the lowest mean CrCl was detected in liver transplant patients shedding JCV (73.93 mL/min) versus BKV and no polyomavirus shedders (81.93 mL/min and 99.08 mL/min, respectively; \( P < .001 \)).

**DISCUSSION**

This investigation revealed different patterns of BKV and JCV shedding by kidney and liver transplant patients and suggests that JCV may have an effect on renal dysfunction in some patients. These findings provide further insights into the pathogenesis of polyomavirus infections following solid organ transplantation.

Overall, 64% of liver transplant patients in the current study shed polyomavirus in the urine compared to 39% of kidney recipients. The majority of liver patients shed JCV at relatively high viral loads. This frequency of JCV excretion is higher than reported in previous studies of liver [33, 34] or lung [31] transplant recipients. These differences may reflect variations in study design, characteristics of the specific patient populations, immunosuppressive regimens received by the patients, the frequency of testing, or variations in sample processing and PCR assay conditions or performance characteristics. However, it is important to note that few studies in liver transplant patients have monitored the shedding of both polyomaviruses. In a cross-sectional study, Randhawa et al [33] found similar frequency of JCV viruria (approximately 22%) and viral load levels in the urines of kidney and liver transplant recipients. That same study reported that BKV was shed more frequently and with higher viral loads in kidney compared to liver transplant patients, as we observed here. In this current study, 1 kidney transplant patient and 5 liver transplant recipients shed both BKV and JCV concurrently, usually on a single occasion. In the earlier study [33], concurrent shedding of BKV and JCV was infrequent in both patient groups and none of the liver transplant recipients had detectable BKV or JCV in the blood. No data on renal function were provided, but JCV was not associated with viral inclusions on histopathologic examination of the allograft among the kidney transplant patients. In a study involving 59 kidney transplant patients in Brazil, 30.5% of recipients were identified as shedding polyomavirus in the urine [34]; BKV and JCV were detected in 27% (16/59) and 3% (2/59) of patients, respectively. Among the polyomavirus-positive kidney transplant patients, 89% (16/18) shed BKV. No data on renal function or kidney allograft histopathology were provided in that report. More recently, a retrospective analysis of urine samples collected during the first year after transplantation from 200 kidney transplant recipients reported detection of BKV and JCV in 35% and 16% of the patients, respectively [35]. The

**Table 3. Quantitative BKV and JCV Viral Loads in Urine of Kidney and Liver Transplant Recipients**

<table>
<thead>
<tr>
<th>Organ Transplant</th>
<th>Log10 Mean Viral Load, copies/mL (range)</th>
<th>Least Squares Mean&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BKV</td>
<td>JCV</td>
</tr>
<tr>
<td>Kidney</td>
<td>5.88 (3.96–9.05)</td>
<td>7.08 (4.03–9.01)</td>
</tr>
<tr>
<td>Liver</td>
<td>5.18 (3.99–6.63)</td>
<td>6.59 (3.02–8.82)</td>
</tr>
<tr>
<td>Least squares</td>
<td>5.20</td>
<td>6.64</td>
</tr>
</tbody>
</table>

Abbreviations: BKV, BK virus; JCV, John Cunningham virus.

<sup>a</sup>Polyomavirus urinary viral loads were higher for kidney transplant recipients than for liver transplant patients (\( P = .043 \)).

<sup>b</sup>Urinary viral loads were higher for JCV than for BKV in both groups of transplant recipients (\( P < .0001 \)).
median viral load in the urine was higher for BKV than JCV. No JCV viremia or nephropathy was observed in that study.

In a longitudinal study of 90 lung transplant recipients monitored over 4.5 years, 59 (66%) had urinary polyomavirus detected at least once [31]. This included 38 (42%) positive for BKV, 25 (28%) for JCV, and 6 (7%) for simian virus 40. Among virus-positive patients, JCV was shed more frequently over time and at higher levels than the other viruses. No correlation was found between polyomavirus infection and renal dysfunction, but BKV was associated with poorer survival.

The present analysis found JCV urinary shedding to be significantly associated with lower CrCl in both kidney and liver transplant patients, suggesting that JCV may be contributing to renal dysfunction in some patients. This finding is consistent with reports that JCV can be linked with some cases of PVAN in renal transplant patients [22–24]. To date, no studies have established a clear association between BKV and renal dysfunction/disease in nonkidney solid organ transplant recipients [31, 36–42]. Data of BKV and renal dysfunction/disease among liver transplant patients are limited [39, 41, 43, 44]. A prospective study of BKV in 62 liver transplant patients showed that BKV was detected in urine and blood in 14.5% and 18% of the patients, respectively [44]. Although no relationship was observed between single episodes of BKV viremia and renal function, 3 liver transplant patients with persistent BKV viremia did display renal dysfunction. In contrast, a cross-sectional study among 41 liver transplant patients suggested there was no relationship between the presence of BKV in the urine and renal function [43]. In a prospective prevalence study of 100 pediatric liver transplant recipients, polyomavirus viruria was detected in 19%, but there was no relationship with kidney function [39]. These observations have led to the hypothesis that an allograft-specific predisposition exists that results in a high frequency of BKV-associated renal dysfunction/disease among kidney transplant recipients, when compared to nonkidney recipients. The underlying mechanism is not known, but it is speculated that impaired immune surveillance of the infected kidney allograft might contribute to an environment favorable for BKV replication. Our findings among liver transplant patients who shed JCV suggest that multiple synergistic factors may be required for the development of renal dysfunction in the native kidney of nonkidney transplant patients.

The influence of immunosuppression on BKV reactivation in kidney transplant recipients has been discussed [45, 46]. Brennan et al [46] evaluated both BK viruria and viremia in a randomized trial comparing cyclosporine to tacrolimus in kidney transplant recipients. All received azathioprine, but mycophenolate was substituted in highly sensitized kidney recipients or in patients with history of gout. Overall, there was no difference in the occurrence of viruria and viremia between the 2 regimens, but the drug combination of tacrolimus–mycophenolate was associated with the highest rates of BKV viruria, viremia, and sustained viremia compared to the cyclosporine–mycophenolate combination [46]. In a recent review of the influence of tacrolimus versus cyclosporine on BKV reactivation, the authors concluded there was an important effect of immunosuppression on reactivation of BKV, but specific immunosuppressive agents could not be identified as having a direct effect on the virus [45]. In previous studies, evaluations of possible direct effects on virus replication were confounded by the uncontrolled addition of immunosuppression to treat rejection.

There are several limitations to our investigation. The study included a population of stable outpatient transplant recipients and this may have introduced a bias, as more complicated patients were not included. We did not have information about the BKV serostatus of kidney donors to correlate with BKV shedding by recipients. We did not perform histopathologic examination of the kidney in patients shedding JCV. We failed to detect JCV viremia in patients with JCV viruria, a finding that might reflect the frequency of sample collections (ie, every 3 months); more frequent sample collection/monitoring might be necessary to correlate JCV viruria and viremia.

In conclusion, our study suggests that there are different patterns of BKV and JCV reactivation and shedding in kidney and liver transplant patients and that JCV may have a role in renal dysfunction in some solid organ transplant recipients. Further studies are warranted to elucidate the mechanisms by which JCV can induce renal disease in both kidney and nonkidney transplant patients.

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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