Evaluation of Fluoroquinolones for the Prevention of BK Viremia after Renal Transplantation

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Background and objectives: Nearly 30% of renal transplant recipients develop BK viremia, a prerequisite for BK nephropathy. Case reports have evaluated treatment options for BK virus, but no controlled studies have assessed prophylactic therapies. Fluoroquinolone antibiotics were studied for prevention of BK viremia after renal transplantation.

Design, setting, participants, & measurements: This retrospective analysis evaluated adult renal transplant recipients with at least one BK viral load (blood) between 90 and 400 days after transplantation. Six to 12 months of co-trimoxazole was used for Pneumocystis prophylaxis. In sulfa-allergic/-intolerant patients, 6 to 12 months of atovaquone with 1 month of a fluoroquinolone was used. Fluoroquinolones can inhibit BK DNA topoisomerase. The two groups studied were those that received 30 days of levofloxacin or ciprofloxacin after transplantation and those that did not. The primary endpoint was BK viremia rates at 1 year. Of note, of the 160 patients not receiving fluoroquinolone prophylaxis, 40 received a fluoroquinolone for treatment of a bacterial infection within 3 months after transplantation. Subgroup analysis evaluating these 40 patients against the 120 who had no exposure to fluoroquinolones was completed.

Results: A 1-month fluoroquinolone course after transplantation was associated with significantly lower rates of BK viremia at 1 year compared with those with no fluoroquinolone. In the subgroup analysis, exposure to fluoroquinolone for treatment of bacterial infections within 3 months after transplantation was associated with significantly lower 1-year rates of BK viremia.

Conclusions: This analysis demonstrates that fluoroquinolones are effective at preventing BK viremia after renal transplantation.


One complication of increasing significance in renal transplant recipients (RTR) is BK polyomavirus. Polyomaviruses belong to a family of small, circularized, double-stranded DNA viruses called Papovaviridae (1–3). Twelve members of the polyomavirus family have been described in a variety of species including mice, monkeys, and humans, with SV40 being the most studied (4). Many studies related to viral replication, assembly, structure, gene expression, and DNA replication have been performed with SV40 and its large T antigen (4). In humans, there are two known pathologic polyoma strains, BK and JC. The majority of healthy individuals are seropositive for antibodies against both viruses. Seroconversion normally occurs in childhood, possibly associated with mild upper respiratory tract symptoms. These viruses rarely are associated with disease in immunocompetent individuals. During periods of immunosuppression the virus is reactivated and can be associated with significant morbidity (1–3).

In RTR, the major diseases caused by BK virus (BKV) are tubulointerstitial nephritis and ureteral stenosis, which occur after BKV reactivates from its latent state with the onset of immunosuppression (3,5,6). BKV causes disease of the genitourinary tract, due in part to its tropism for genitourinary epithelium. BKV-induced nephropathy (BKVN) presents with evidence of allograft dysfunction, resulting in either an asymptomatic acute or a slowly progressive rise in serum creatinine concentrations (1–3,5,6). Some studies have reported the incidence of BK viremia to be as high as 29% (7). BK viremia is believed to be a precursor to BKVN with BK viremia preceding nephropathy by 1 to 12 weeks (5,6,8–10). One analysis showed a peak in BK viremia occurring at 3 months after transplantation (11). The onset of BKVN occurs at a mean period of 9 to 12 months after transplantation; however, some cases have been reported as early as 7 days after transplantation (1–3,5,6). It is estimated that BKVN affects up to 10% of RTR, frequently resulting in permanent renal dysfunction or allograft loss (5,6). The temporal relationship between the introduction of more potent immunosuppressive agents and BKV has led to the proposal that intensity of immunosuppression is a risk factor for BKVN.
Other possible risk factors include increased age, male gender, Caucasian race, diabetes mellitus, and acute rejection (12–14). Some donor-related risk factors are the presence of active BKV or cytomegalovirus (CMV) infection and deceased donor versus living donor transplant (12–14).

Currently, treatment options for BKV are limited and management recommendations are formulated on the basis of individual case reports and small case series. Pharmacologic options with activity against BKV are limited; therefore, a reduction in the degree of immunosuppression in patients with BK viremia is often thought to be the first-line option to prevent BKVN (1–3). This strategy focuses on routine patient monitoring for development of BK viremia and then on reducing immunosuppression upon diagnosis. However, in immunosuppressive regimens utilizing drug-minimization or -withdrawal strategies, it may be inconceivable to reduce immunosuppression in some patients without increasing the risk of acute rejection. Therefore, the search for a pharmacologic option for the prevention and management of BK infection remains a priority.

Despite the lack of a directed antiviral intervention, there are several agents that have anti-BKV activity, including intravenous immune globulin (IVIG), cidofovir, leflunomide, and the fluoroquinolone antibiotics (6,15,16). All of these agents have been reported to provide some benefit in managing BKV in anecdotal cases. It should be noted that these cases have been complicated by the fact that administration of the antiviral agent was also done simultaneously with immunosuppression reductions, making it difficult to comment on the true effectiveness of the pharmacologic intervention. Beyond the paucity of data and the poor quality of the existing evidence, there may be several reasons why the use of IVIG, cidofovir, and leflunomide are not ideal in RTR. The use of IVIG is limited by its expense, frequent nationwide shortages, potential for nephrotoxicity from sucrose-containing formulations, and lengthy administration times that may necessitate hospital admission (16).

The active metabolite of leflunomide, A771726, has wide interpatient variability and also has a long half-life (mean = 15 days). Therapeutic drug monitoring is suggested when using leflunomide, but the limited availability of A771726 levels may preclude its routine use at some institutions. This agent is also associated with significant hematologic and hepatic toxicities (17).

Cidofovir is highly nephrotoxic and, on the basis of in vitro data, appears to have minimal antiviral activity against BKV (18,19). Clinically, the fluoroquinolone antibiotics may represent a class of medications that could manage BKV infections with a low incidence of adverse events at a reasonable expense. Fluoroquinolones display anti-BK properties through inhibition of DNA topoisomerase and polyomavirus associated large T-antigen helicase (2,16). An in vitro analysis using older fluoroquinolone compounds, nalidixic acid and oxolinic acid, demonstrated that these agents are capable of inhibiting BKV DNA replication (20). In another analysis using contemporary fluoroquinolones (i.e., levofloxacin, trovafloxacin, ciprofloxacin, ofloxacin, and gatifloxacin), it was shown that these antibiotics have the ability to inhibit viral DNA replication and T-antigen helicase activity of SV40 (21). These agents demonstrate the ability to inhibit viral replication and block the cytopathic effect of SV40 virus in permissive monkey cells. Given the homology among SV40, BK, and JC T-antigens, these data could be applied to management of BKV. In a clinical analysis of RTR, 7 of 10 patients with active BKV replication had a reduction in viremia or urinary decoy cells 2 months after receiving a 10-day course of gatifloxacin without any reduction in immunosuppression (6,22).

Given the literature suggesting the activity of fluoroquinolones against BKV, we hypothesize that patients receiving a 1-month postoperative course of fluoroquinolones would have a lower frequency of BK viremia than those patients not receiving this therapy. To test this hypothesis, we compared the rates of BK viremia in patients who were exposed with those who were not exposed to fluoroquinolones for posttransplant prophylaxis.

Materials and Methods

Study Design

This is a single-center, retrospective study of a cohort of patients undergoing renal transplant. Consecutive RTR from January 1, 2004, to December 31, 2008, were identified for review. The study was designed to include patients aged 18 years or older and those that received at least one blood BK viral load sample between 3 and 13 months after transplantation. Patients were excluded if they were desensitized before the transplant (i.e., patients with preformed antidonor antibodies undergoing pretransplant immunomodulation). The analysis used was based on intention to treat for prevention of BK viremia.

This study was approved by our institutional review board as a retrospective analysis; therefore, informed consent was not required. Inpatient and outpatient medical records, including clinic visit notes, laboratory data, and medication histories, were reviewed for demographics, transplant characteristics, laboratory results, BK viral loads, immunosuppressive therapies, and patient and allograft outcomes. Of note, all patients transplanted at our institution received their long-term follow-up at our institution.

Patients and Intervention

We used 6 to 12 months of sulfamethoxazole/trimethoprim (SMZ/TMP) for Pneumocystis jirovecii pneumonia (PCP) prophylaxis after renal transplantation. In patients with sulfal-allergies, history of intolerance to SMZ/TMP, or glucose-6-phosphate dehydrogenase deficiency, 6 to 12 months of atovaquone was used. Unlike SMZ/TMP, atovaquone has a limited antimicrobial spectrum of activity; therefore, we co-administered 1 month of a fluoroquinolone with atovaquone. The fluoroquinolone was added for urinary tract and incision site prophylaxis. The two study groups were based on the PCP prophylaxis regimen they received:

**Group I.** All patients in this group received SMZ/TMP prophylaxis for 6 to 12 months \((n = 160)\).

**Group II.** All patients in this group received atovaquone prophylaxis for 6 to 12 months, along with 1 month of a fluoroquinolone antibiotic, either ciprofloxacin (250 mg twice daily) or levofloxacin (250 mg daily), starting on postoperative day 1 \((n = 25)\). All doses were adjusted to renal function.
**Clinical Definitions**

Clinical practice with reference to the diagnosis and management of BKV was similar between the groups. We used a pre-emptive strategy to detect BKV DNA by using blood PCR tests at 1, 3, and 6 months after transplantation; however, other tests may have been added in those patients where there was clinical suspicion of BKV. Patients were considered to have BK viremia if their viral load was ≥500 copies/ml. The diagnosis of BKVN was made by biopsy after immunostaining for polyomavirus antigens and/or pathologist-reported virus-mediated tubular epithelial cell damage and corresponding inflammation. Follow-up days were censored for the last BK viral load completed. All patients included in this analysis were followed in our clinics for at least 1 year. Mean follow-up time was 400.9 ± 278 days in Group I and 346.6 ± 213 days in Group II ($P = 0.03$).

**Immunosuppressive Regimen**

All patients received induction immunosuppression using either antithymocyte globulin rabbit (r-ATG) or basiliximab, and were initially treated with tacrolimus, mycophenolate mofetil (MMF) and corticosteroids. However, most patients were eligible for rapid steroid withdrawal, where corticosteroids are withdrawn within the first few weeks after transplantation. Tacrolimus dose adjustments were made to achieve target trough concentrations of 8 to 12 ng/ml for the first 6 months after transplantation and 5 to 10 ng/ml thereafter. The initial MMF dose was 2 g/d with a goal of maintaining this dose throughout the first 12 months. Dose adjustments were made in the presence of severe myelosuppression or gastrointestinal intolerance. Methylprednisolone was started in the operating room at a dose of 500 mg before graft reperfusion, followed by 200 mg in two divided doses after the procedure on postoperative day 0 and quickly tapering the dose over the first 5 postoperative days to prednisone 20 mg/d. In patients eligible for rapid steroid withdrawal, corticosteroids were completely withdrawn once tacrolimus trough concentrations were >8 ng/ml. All patients receiving this protocol had their corticosteroids completely withdrawn within 3 weeks of the transplant. In patients ineligible for rapid steroid withdrawal, prednisone doses were lowered to a maintenance level of 2.5 to 5 mg/d within 2 months of the transplant procedure. Acute rejection was treated using intravenous pulse methylprednisolone at a dose of 250 to 500 mg/d for 3 days, depending on the severity of the episode. In cases of steroid-resistant rejection, treatment with r-ATG 1.5 mg/kg per day for 4 to 14 days was used.

**Statistical Analysis**

Categorical variables were analyzed using the Fisher’s exact test. The $t$ test was used to compare means of continuous variables. Differences were considered significant at $P < 0.05$. Statistical analysis was performed using GraphPad InStat 3.0 (GraphPad Software, San Diego, CA) and SAS 9.2 (SAS Institute Inc., Cary, NC).

**Results**

**Patient Characteristics**

The baseline demographic data and transplant characteristics were similar between the two groups (Table 1). The type and degree of clinical immunosuppression were also similar between the groups, as were the transplant-related outcomes (Table 2). In terms of posttransplant outcomes, the incidence of biopsy-proved acute rejection (BPAR), anti-body-mediated rejection (AMR), and steroid-resistant rejection were comparable between the groups.

**Frequency of BK Viremia**

When the primary endpoint, the incidence of BK viremia within 1 year of transplant, was evaluated, it was shown that there was a significantly higher rate of BK viremia in those patients who did not receive a 1-month fluoroquinolone course (Group I = 36 [22.5%] versus Group II = 1 [4%]; $P = 0.03$). As for the precision of the estimates, the 95% two-sided confidence interval around the proportion in Group I is (16.03%, 28.97%), and in Group II it is (0%, 11.68%), showing that the sample sizes were sufficiently large for adequately precise estimates. In addition, the samples sizes of 25 treatment patients and 160 control patients provide 80.2% power to reject the null hypothesis of equal BKV proportions. Evaluation of some of the secondary BKV outcomes showed similar results among the groups (Table 3). The one patient that developed BK viremia in Group II did so 45 days after transplantation and had a peak viral load of 22,800 copies/ml. In Group I, the mean onset of BK viremia was 181 ± 153.7 (range: 26 to 733) days with a median viral load of 68,100 (range: 600 to 36,000,000 copies/ml). Of the patients with diagnosed BK viremia, 20 of 40 patients in Group I (50%) and none in Group II ($P = ns$) had continued viremia at the conclusion of the analysis. The overall incidence of BKVN was 35% in Group I and 100% in Group II ($P < 0.0001$ using binomial test). Four of the 14 patients in Group I that developed BKVN went on to lose their allografts due in part...
to BKV. All four of these patients had persistent BKV-related disease noted on biopsies. In one patient, a transplant ne-
phrectomy was completed and the pathologist confirmed BKVN as a contributing factor to this patient’s allograft failure. In contrast, the patient that developed BKVN in Group II did not suffer allograft loss.

The fluoroquinolones are commonly prescribed antibiot-
cics. Realizing that some patients in Group I might have been administered a fluoroquinolone to treat a bacterial infection, we evaluated the effect of short-term fluoroquinolone ther-
apy. In Group I, 40 (25%) patients were administered a fluoroquinolone within the first 3 months after transplanta-
tion to treat a bacterial infection. In these 40 patients, we found that the incidence of BKV at 1 year after transplanta-
tion was 7.5% compared with 27.5% in patients not treated with a fluoroquinolone (P = 0.008). As for the precision of these estimates, the 95% two-sided confidence interval around the proportion within the control group is (22.54%, 39.06%), and for patients who received a fluoroquinolone it is (0%,15.66%), showing that the sample sizes were suffi-
ciently large for adequately precise estimates. In addition, the samples sizes of 40 treatment patients and 120 control patients provide 92.6% power to reject the null hypothesis of equal incidence BKV proportions. Evaluation of the second-
ary BKV outcomes is shown in Table 4.

Discussion

BKV continues to be a major problem among RTR, despite pre-emptive strategies aimed at early viral detection. Pre-
emptive strategies are based upon the idea that periodic monitoring of BK viral loads allow for the diagnosis of early

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<th>Table 2. Immunosuppression and transplant outcomes</th>
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<td>Mean tacrolimus concentration (ng/nl; mean ± SD)</td>
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<td>Mean mycophenolate dose (mg/day; mean ± SD)</td>
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<td>Rapid corticosteroid withdrawal</td>
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<td>Biopsy-proven acute rejection</td>
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<td>Mean WBC (1000/mm³; mean ± SD)</td>
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<td>Incidence of CMV disease</td>
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<th>Table 3. BKV-related outcomes</th>
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<td>BK viremia at 1 year (±1 month)</td>
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<td>Serum creatinine at 12 months (mg/dl; mean ± SD)</td>
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<td>Allograft loss secondary to BKV</td>
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ns, not significant.
systemic infections. This facilitates prompt treatment, which may limit morbidity. One significant drawback of pre-emptive strategies is that success requires vigilance and adherence to monitoring protocols, whereas another is that the cost of screening is not universally covered by health insurance. A better approach to the management of BKV may be to emulate strategies used to manage CMV and PCP. It has been reported that in RTR not receiving routine antimicrobial prophylaxis the incidence rates of CMV disease and PCP are 20 to 60% and 5 to 15%, respectively (23,24). Given the high incidence of each of these infections, antimicrobial prophylaxis is commonplace for the prevention of disease caused by these organisms. With the knowledge that the incidence of BK viremia after renal transplantation can be as high as 30%, using strategies aimed toward the prevention of BK viremia would benefit this population.

To our knowledge, this is the first analysis evaluating a pharmacologic intervention versus a control group for the prevention of BK viremia after renal transplantation. In a study by Koukoulaki and colleagues, 32 RTR were evaluated for the effect of prophylactic ciprofloxacin on BKV replication (25). In this analysis, all patients received a 10-day course of a third-generation cephalosporin followed by ciprofloxacin (mean dose = 703.1 ± 249.5 mg/d) for 3 weeks (n = 9) or 6 weeks (n = 23). The incidence of posttransplant BK viruria and viremia was monitored at postoperative months 0, 1, 3, 6, 9, and 12. An increasing tendency of viruria and viremia was noted, which peaked at month 3, declining thereafter. It should be noted that although the incidence of BK viruria and viremia seemed high, the lack of a control group makes it impossible to evaluate the effectiveness of ciprofloxacin prophylaxis (25). In contrast, our analysis revealed that the use of a 1-month course of fluoroquinolones immediately after the transplant procedure was associated with a significant reduction in the incidence of BK viremia.

A prophylaxis strategy using the fluoroquinolone antibiotics has been evaluated in hematopoietic stem cell transplant patients. In this analysis, hematopoietic stem cell transplant recipients receiving ciprofloxacin prophylaxis demonstrated a decrease in BK viruria and significantly reduced rate of hemorrhagic cystitis (0 of 39 recipients versus 8 of 29 recipients not treated with ciprofloxacin) (26).

As mentioned earlier, there are several risk factors that have been identified that increase the incidence of BK viremia, including patient age, male gender, diabetes, Caucasian race, and acute rejection. The prevalence of these risk factors was similar between the two groups. The degree of posttransplant immunosuppression has also been described as a risk factor for development of BKV. In our analysis, r-ATG induction therapy was used most frequently in both groups. In terms of maintenance immunosuppression, tacrolimus trough concentrations were nearly identical at months 3, 6, and 12 after transplantation. MMF doses were numerically higher in Group I, but this difference did not meet statistical significance at any time point. Also, there were six patients with steroid-resistant BPAR in Group I and none in Group II; however, in the six patients that developed steroid-resistant rejection, none developed BK viremia. The incidence of AMR was also numerically higher in Group I, but not statistically different compared with that in Group II. Of the 13 patients with AMR in Group I, only 2 patients developed BK viremia during the study period. Despite the need for increased immunosuppression in this small number of patients in Group I, there appeared to be little influence on the development of BK viremia. In addition to evaluation of the individual risk factors, we also used a propensity score, which is a joint measurement of all background variables. Analysis by propensity score showed that a composite of these variables had no effect on the incidence of BKV in Group I versus Group II.

We acknowledge the limitations of our study. Data were collected at a single center and done so in a retrospective manner. There was a small sample size in the fluoroquinolone group. We were able to capture short-term use fluoroquinolone antibiotics in Group I when the agent was written by one of our institution’s prescribers. However, we could not account for the routine use of the fluoroquinolone antibiotics when orders were written by prescribers located outside of our institution, which may have influenced the incidence of BK viremia. A complete evaluation of fluoro-
quinolone-induced adverse events and their potential effect on bacterial-resistance patterns was also not assessed. Although the antimicrobial mechanism of action of atovaquone does not lend itself to efficacy versus BKV, we cannot rule out the potential effect that this medication may have played in suppressing BK viremia. However, exposure to atovaquone would not explain the differences in BK viremia rates seen in the subgroup analysis. These are all factors that need to be incorporated in a prospective analysis.

One concern with routine BKV screening may be the incidence of delayed onset of viremia occurring after the screening period. Although BK viral loads were checked upon clinical suspicion of BKV infection, there may have been some patients in both groups that developed BK viremia after 6 months that did not receive a follow-up viral load. However, a similar percentage of patients in both groups received at least one viral load test after 6 months (Group I = 73.8% versus Group II = 64%; P = ns). This should help confirm that cases of BK viremia were not missed in either group.

Conclusions
The prophylactic use of the fluoroquinolones for the prevention of BK viremia represents a major departure from the normal thought process in managing RTR. The potential effect on transplant outcomes would be significant. We are currently undertaking a randomized, double-blind, placebo-controlled trial to definitively prove if fluoroquinolones are effective in prevention of BKV infection postrenal transplantation.

Disclosures
None.

References


Access to UpToDate on-line is available for additional clinical information at http://www.cjasn.org/