BK Virus Nephritis after Renal Transplantation

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BK virus nephritis is an increasing problem and is posing a threat to improving renal transplant graft survival. The pathogenesis of this condition remains to be investigated. Higher prevalence of BK virus infection in recent years has been correlated with declining acute rejection rates and the use of potent immunosuppressive agents. Patients with this infection usually have asymptomatic viremia and/or nephritis with or without worsening of renal function. The diagnosis of this disease is based on detecting the virus or its effects in urine, blood, and renal tissue. In the past, approximately 30 to 60% of patients with BK virus nephritis developed graft failure. In recent years, the combination of early detection, prompt diagnosis, and therapies including preventive measures have resulted in better outcomes.


The term “BK” originated from a patient’s initials, in whom it was first detected in 1971 (1). The next observed case was published by investigators from the University of Pittsburgh in 1995 (2). Since then, there have been numerous reports on BK virus (BKV) infection and BKV nephritis (BKVN) in renal transplant recipients (3–8). The factors that lead to its higher incidence in recent years and its pathogenesis remain poorly understood. Increased awareness, the ability of clinicians to recognize this infection, and the availability of better diagnostic tools may be contributing to higher prevalence of this disease in recent years (9). The use of potent immunosuppressive combination therapy with mycophenolate mofetil (MMF) and tacrolimus has been thought to play a role in the occurrence of this infection (10–12); however, this infection is also seen with cyclosporine and sirolimus therapy (13). Prevalence of BK viremia within 1 yr after transplantation is approximately 20% (6,14) and is higher than the prevalence of acute rejection of 13% reported for the year 2003 (15). This review discusses the pathogenesis, clinical features, therapy, and the short- and long-term renal graft survival with reference to BKV infection.

Pathogenesis of BKV Infection
Potential factors that contribute to the pathogenesis of BKVN may be a combination of (1) ineffective immune surveillance by the host T lymphocytes, (2) the absence of previous humoral immunity to BKV, (3) molecular sequence variability of the virus, and (4) alloimmune activation. These details have been reviewed elsewhere (9) and are also discussed next.

Cellular immunity in the development and clearance of BKV infection has remained an important area of research in the past several years. Comoli et al. (16) showed a reduction in BKV-specific IFN-γ-secreting lymphocytes in patients with BKVN compared with healthy control subjects. The authors noted an increase in patient IFN-γ-secreting lymphocyte levels with reduction in immunosuppressive therapy similar to that of their healthy counterparts (16). Prosser et al. (17) used an IFN-γ enzyme-linked immunosorbent spot (ELISPOT) assay to measure cellular immune response directed against BKV large T antigen in patients with BKVN at the time of diagnosis and after full resolution of infection. A robust 400% increase in IFN-γ activity was noted with resolution of BKVN.

Within the viral genome, both the large T antigen and VP1 gene products have been shown to contain epitopes that are responsible for CD4+ and CD8+ cell recognition (18–25). Within this body of work, Provenzano et al. (25) showed that specific sites within the large T antigen p53 binding region elicited increased CD8+ T cell responses. Chen et al. (19) showed two epitopes within the VP1 capsid protein that were recognized by cytotoxic T lymphocytes. These regions were found to be variably recognized in healthy individuals compared with kidney transplant recipients. Stronger T cell response was associated with lower viral load, whereas a weaker response was associated with higher viral load and viral persistence. Thus, regions in both the VP1 and large T antigen gene products contain conserved sequences that likely are responsible for cellular immunity against BKV (18–25). Leuenberg et al. (21) hypothesized that the BKV agnoprotein would also contain epitopes that would stimulate T cell activity. This hypothesis stemmed from the observation that viral agnoprotein production is high after infection. Their results, using an ELISPOT assay, showed little IFN-γ production in both healthy volunteers and kidney transplant recipients when stimulated by agnoprotein. It is interesting that they showed no increase in anti-agnoprotein Ig production in patients with BK viremia. Ig activity against large T antigen and VP1 was increased in patients with BK viremia compared with healthy control subjects (21).

Humoral immunity may play a role in the pathogenesis of BKVN because those with previous immunity to BKV may not develop clinical infection, irrespective of the number of circulating viral copies. Bohl et al. (26) found that recipients of a kidney from a seropositive donor were more likely to develop
BK viremia compared with those who received a kidney from a seronegative donor. Recipient serostatus did not show a statistically significant difference in viremia, however. In contrast, Smith et al. (27) found recipient seronegativity to be a significant risk for development of BKVN \( (P = 0.01) \) in a pediatric population. Donor serostatus was not analyzed because of paucity of data from all donors. Ginevri et al. (28) showed recipient seronegative status and MMF use at baseline to be predictive of BKV infection \( (P < 0.005) \). In their retrospective analysis of pediatric renal transplant recipients’ donor serostatus, cold ischemia time, acute rejection history, use of calcineurin inhibitor, and induction agent used were not contributing factors to posttransplantation BKV infection. The role of the recipient’s humoral immunity to BKV at the time of transplantation and at viremia to the development of BKVN needs further evaluation.

Occurrence of BKVN in renal transplant recipients as opposed to liver and heart transplant recipients unveils a potential role of alloimmune activation in renal grafts with BKV activation and frank nephritis. Awadalla et al. (29) showed that the occurrence of BKVN correlates with a higher degree of HLA mismatches, which postulates the role of alloimmune activation. Investigators from Emory University (30) showed using a mouse polyoma transplant model that polyoma viral nephritis occurs only in the presence of alloimmune activation. Thus, subclinical alloimmune activation in renal grafts may trigger BKV replication and nephritis and explains why this is specific to renal grafts. It is interesting that Drachenburg et al. (31) showed an inverse relationship between the level of HLA matches and graft survival in patients with BKVN. Patients who maintained graft function had lower mean HLA match of 1.5 as opposed to 2.87 among those who lost their graft \( (P = 0.001) \), thus postulating lack of HLA matches as a predictor of better outcome in patients with BKVN. The authors speculated that antiviral immune response is likely to trigger a variety of nonspecific inflammatory and fibrogenic mechanisms that lead to graft failure, and lack of HLA donor–recipient matching may result in the inability of the host to mount an efficient specific antiviral immune response. Thus, lack of HLA matches may be beneficial in patients with BKVN.

In addition, viral entry into susceptible host cells is an integral component of BKV infection. Eash et al. (32) showed that the caveolin-1 scaffolding domain and presence of cholesterol were required for viral entry into an immortalized primate renal tubular cell line (Vero). Recently, Moriyama et al. (33) showed that blockade of caveolin-induced endocytosis, either by direct inhibitors or via small interference RNA depletion of caveolin-1, caused significant decreases in BKV infectivity as measured by immunofluorescence. They also proved that blockade of clathrin-mediated viral entry had no effect on infectivity. BKV particles were also found to co-localize with caveolin-1, not clathrin. It therefore seems that BKV entry into its human target cells in vitro depends on a caveola-dependent pathway. This has potential future implications regarding therapeutic options in patients with BKV infection. Thus, the pathogenesis of BKV infection is possibly secondary to a combination of cellular and humoral immune deficiencies with alloimmune activation as well as BKV’s tropism to renal tubular epithelial cells.

### Clinical Features

#### Clinical Presentation

BKVN has increasingly been recognized as an early event and occurs within the first year after transplantation (4,6,11,34,35). Patients with BKVN usually remain asymptomatic and are detected when they experience renal insufficiency. BKV DNA is detected in the urine and plasma in nearly all cases of BKVN (35). Renal dysfunction secondary to ureteric stricture leading to hydronephrosis is occasionally seen (36), and severe systemic disease leading to multiorgan failure has been reported (37).

#### Diagnosis

Decoy cells seen on urine cytology have been observed in patients with BKVN and originate from infected renal tubular cells with nuclei altered by viral inclusions. The presence of decoy cells is a sensitive (100%) measure but has a low positive predictive value of 29% for the diagnosis of BKVN (38). Overall, it seems that the presence of decoy cells is a good screening test but not diagnostic of BKVN.

Quantification of viral load in blood and urine with either viral DNA or mRNA to VP-1 has been used to diagnose BKVN. Urinary BKV DNA is seen in at least 50% of transplant recipients, but variability in testing conditions has created difficulty in standardizing this method for definite diagnosis (39). Some authors have suggested using urinary VP-1 mRNA load to document active viral replication (40). BKV DNA quantification in plasma is used as an important diagnostic tool and is detected in 15 to 30% of renal transplant recipients during the first posttransplantation year (6,14). Quantification of BKV DNA in plasma by PCR has sensitivity and specificity of 100 and 88%, respectively, to BKVN (34,38); however, not all patients with BK viremia have nephritis (positive predictive value of 50%) (39). Higher BKV DNA copy number is associated with an increased likelihood of having nephritis and has also been correlated with severity of disease (39). BK V DNA >7000 or 10,000 copies/ml plasma is used by some as a threshold for significant infection and correlates with BKVN. Nephritis, however, can be seen with BKV DNA <7000 copies/ml plasma (9,39).

Limaye et al. (41) reported BKVN in an immunosuppressed nonrenal transplant patient without detectable viremia. The exact reason for this is unclear, and possibilities include nonstandardization of BKV DNA estimation leading to variability in levels of viremia from one laboratory to another. Thus, one should be cautious in recommending a level of viremia as a threshold for the occurrence of nephritis.

Pathologic findings of infection include viral cytopathic changes in the epithelium of tubules, glomeruli, and collecting ducts with interstitial inflammation and varying degrees of tubular atrophy or fibrosis (42). Patchy interstitial involvement is common early in the course of disease followed by extensive inflammation progressing to fibrosis. Sampling error as a result of the focal nature of renal involvement does pose a problem in considering renal histology as a gold standard for the diagnosis.
of infection (42). The presence of tubular basement membrane immune deposits (positive C4d staining) has been seen in some cases and is associated with more severe disease (43,44). An additional impediment to diagnosis of BKVN on biopsy specimens is its similarity to acute rejection. Immunoperoxidase staining for SV40, another member of the Papovoviridae family, cross-reacts with BKV and highlights infected tubular cells, suggesting virus-induced inflammation as opposed to acute rejection (9,35). Another method used to delineate between acute rejection and BKVN is immunohistochemical staining of renal tissue or urinary sediment with anti–HLA-DR, which has been shown to be associated with acute rejection (45–48). In addition, a higher proportion of CD20+ cells in the infiltrates in the kidney has been correlated with BKVN as opposed to acute rejection (4). A renal biopsy grading system for BKVN has been implemented (42). Biopsies that display minimal or mild viral cytopathic changes are designated with an A grade; increased inflammation and mild to severe viral cytopathic changes are given a B grade; and C grade biopsies have moderate to severe tubular atrophy, interstitial fibrosis, and inflammatory infiltrates (42). The diagnosis of BKV infection is based on quantification of plasma and/or urinary BKV DNA with presence of urinary decoy cells in urine cytology with or without renal histologic features of nephritis.

**Treatment**

The goal in treating BKV infection is to eliminate the virus while preserving renal function and preventing acute or chronic rejection. The treatment of BKV infection has centered on alterations in immunosuppressive therapy with or without antiviral therapies. Several regimens for altering immunosuppressive therapy have been attempted to treat this infection. These include discontinuation of an agent, decreasing an agent, switching immunosuppressant within the same class or to another class, and steroid avoidance. As a proof of this strategy, patients who had BKV infection and were inadvertently treated with an antilymphocyte agent or pulse corticosteroids for presumptive acute rejection had rapid progression toward graft failure (6,49).

Discontinuation of a single immunosuppression agent, antimetabolite (MMF or azathioprine), upon recognition of viremia has been used successfully to clear viremia (49). Reduction in immunosuppression by halving both antimetabolites and calcineurin inhibitors has also been successful in eliminating viremia and preserving renal function (50). Steroid avoidance has been suggested to decrease the prevalence of BKV infection (51).

Antiviral therapy with leflunomide or cidofovir has been used in conjunction with decreasing immunosuppression in some cases. Leflunomide is an immunosuppressant medication developed for use in treatment of rheumatoid arthritis. A metabolite of leflunomide (A77 1726) has been shown to have antiviral properties (52,53). Treatment with this agent is associated with decreasing circulating viral copies (53–55); however, introduction of leflunomide has been attempted only with concomitant discontinuation of the antiproliferative agent MMF and decreased dosages of tacrolimus (53,54). Hence, it is unclear whether viral clearance is secondary to leflunomide or the decrease in immunosuppression. Leflunomide treatment is limited because of the requirement of large doses of drug, necessity for liver function monitoring to detect liver toxicity, and need for therapeutic monitoring of trough A77 1726 levels for effectively treating this infection (53–55). In their successful report of 17 patients with BKVN, Williams et al. (53) showed viral clearance or reduction in viral load when A77 1726 levels persisted above 40 μg/ml. This requirement for a target therapeutic level of leflunomide was confirmed by the same investigators in their subsequent study of 26 patients with BKVN (54). A short-acting leflunomide, FK778, has been compared with reduction in immunosuppression alone in a prospective, randomized study to treat BKVN. This study did not show any beneficial effect of FK778 in clearing the virus as opposed to reduction in immunosuppression alone (A. Guasch, Department of Medicine, Emory University School of Medicine, Atlanta, Georgia, personal communication (May 9, 2007 at the American Transplant Congress), presented for Astella Study Group ATC 2007).

Cidofovir, a nucleoside analogue used in the treatment of cytomegalovirus retinitis, has shown activity against BKV. Unfortunately, cidofovir is nephrotoxic, and its use must be weighed against the possible risk for further worsening of renal function (56–58). Cidofovir has also been used in conjunction with lowering immunosuppression, making comments regarding its efficacy difficult. A prospective, randomized, controlled trial is under way to evaluate the efficacy and safety of cidofovir for patients with BKVN (http://www.clinicaltrials.gov/ct2/show/NCT00138424). Cidofovir is administered when patients fail to respond to reduction in immunosuppressive therapy and is administered typically 10 to 20% of that needed in the treatment of cytomegalovirus retinitis in patients with HIV infection (0.25 to 1 versus 3 to 5 mg/kg) (57,58).

Treatment with the anti-CD20 mAb rituximab was recently reported with promising results. Patients who had BKVN and were treated with rituximab and followed for 17 mo had no graft failure compared with 46% graft loss in the control group (59). The administration of intravenous Ig (IVIG) with concomitant reduction in immunosuppressive therapy has been successful; however, efficacy of IVIG is unclear, because it has been administered with concomitant reduction in immunosuppressive therapy (54,60). Wadei et al. (61) recently reported on their experience with treatment of BKVN with cidofovir and IVIG in patients whose immunosuppression was generally reduced in all and converted to cyclosporine therapy in some. Their findings suggested no benefit with conversion to cyclosporine from tacrolimus, use of cidofovir, or IVIG therapies.

Close monitoring for BKV DNA and renal function with any therapy is critical to improving outcome for patients with BKV infection. Elimination of BKV DNA occurs during a period of 6 mo with either an antiviral agent or reduction in immunosuppressive therapy (50,53). At our center, we follow quantitative plasma BKV DNA and renal function every 2 wk for 8 wk then monthly thereafter until clearance of BK viremia and stabilization of renal function. We have been successful in eliminating circulating virus and preventing further renal dysfunction with
low rates of acute rejection (50). Thus, close follow-up is of paramount importance in effectively treating patients with BKVN.

Prevention

Inability to pinpoint the pathogenesis of BKV infection and lack of safe and effective antiviral therapy has prompted investigators to consider a preventive approach in managing this disease. Preventive strategies include identification of this disease by detecting BKV DNA in blood or urine and preemptive reduction in immunosuppression for patients with viremia or viruria. Higher prevalence of viruria, as opposed to viremia, and lack of good correlation with viruria have prompted investigators to use viremia as a better marker for preemptive reduction in immunosuppressive therapy. Vigorous posttransplantation screening and preemptive reduction in immunosuppressive therapy have decreased the prevalence, detected disease at an early stage, and improved graft survival of patients with BKVN at our center (62).

Multiple authors have recommended a step-wise approach to screening for BKV infection. Hirsch et al. (39) recommended initial evaluation of urine cytology (for decoy cells), viruria, or urine VP-1 mRNA load at 3-mo intervals up to 2 yr or if renal dysfunction occurs. A positive screening test is followed by quantification of DNA load in the urine (threshold >10^7 copies/ml), urine VP-1 mRNA (threshold >6.5 x 10^5 copies/ng total RNA), or plasma DNA load (threshold >10^7 copies/ml). If one or more of these tests is above the threshold value, then an allograft biopsy is performed. Screening for quantitative BKV DNA in plasma at 1, 3, 6, 12, and 24 mo after transplantation has been effective in detecting early infection before the occurrence of nephritis (62).

Buehrig et al. (63), among others, proposed use of surveillance allograft biopsies to diagnosis BKVN. Biopsies were performed at posttransplantation month 3 or 4 and at 12 mo detected 18 patients with BKVN within a 5-yr period (1996 to 2001). BKVN was diagnosed in eight patients at the time of surveillance biopsy and 10 patients during nonsurveillance. Their results showed significant improvements in graft status 6 mo after biopsy (P = 0.004) and more favorable histologic appearance at time of biopsy (P = 0.01) in the surveillance group. A trend toward earlier diagnosis of BKVN in the surveillance group was noted (63). More recently, Khamash et al. (64) reported superior graft survival with surveillance allograft biopsy compared with nonsurveillance in a cohort of 74 patients with a mean follow-up of 4 yr. Thus, screening for BKV infection by urine cytology, urinary or plasma BKV DNA, or renal biopsy is effective in identifying early disease.

A strategy for preemptive decrease in immunosuppression is backed by data suggesting that the presence of viruria predates viremia, which in turn predates the detection of clinical renal disease and histologic evidence of BKVN (14,34,38,65,66). Preemptive reduction in immunosuppressive therapy for those with significant viremia has substantially lowered the prevalence of BKVN at our center (62). Thus, detection of early disease and modification immunosuppressive therapy are effective in treating this infection.

Risk Factors for the Occurrence of BKVN

Various risk factors for the occurrence of BKVN are shown in Table 1. These include donor–recipient-specific humoral immunity, alloimmune activation, and immunosuppressive agents. Before 1995, BKVN was rarely identified, and MMF and tacrolimus were introduced at approximately the same period for

<table>
<thead>
<tr>
<th>Year</th>
<th>Total Patients</th>
<th>BKVN (n [%])</th>
<th>Risk Factors</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1997 to 2000</td>
<td>444</td>
<td>40 (4)</td>
<td>HLA mismatches</td>
<td>Awadhala et al. (29)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Previous acute rejection</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>Use of antilymphocyte therapy</td>
<td></td>
</tr>
<tr>
<td>1997 to 2002</td>
<td>100</td>
<td>3 (3)</td>
<td>Recipient’s humoral deficiency (BKV IgG) MMF use at baseline</td>
<td>Ginevri et al. (28)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>2000 to 2004</td>
<td>1027</td>
<td>74 (7)</td>
<td>Recipient age</td>
<td>Khamash et al. (64)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Recipient age &gt;55</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Donor female</td>
<td></td>
</tr>
<tr>
<td>1999 to 2001</td>
<td>286</td>
<td>9 (3.1)</td>
<td>Recipient race (white)</td>
<td>Rocha et al. (70)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Recipient gender (male)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Increase tacrolimus level</td>
<td></td>
</tr>
<tr>
<td>1984 to 2002</td>
<td>173</td>
<td>6 (3.5)</td>
<td>Recipient seronegativity</td>
<td>Smith et al. (27)</td>
</tr>
<tr>
<td>1996 to 2003</td>
<td>1001</td>
<td>41 (4)</td>
<td>No risk factors</td>
<td>Vasudev et al. (67)</td>
</tr>
</tbody>
</table>

*Race and gender (recipient); age and race (donor); cold ischemia time, panel-reactive antibodies, previous transplant, cadaver versus living donor, and kidney versus kidney-pancreas (transplant); and delayed graft function, use of IL-2 receptor blocker, and maintenance immunosuppression cyclosporine versus tacrolimus (posttransplantation) were not identified as risk factors for the occurrence of BKVN.
clinical transplantation; however, occurrence of BKVN does not seem to be limited to the use of specific immunosuppressive agents but may be related to overall degree of immunosuppression. Brennan et al. (14) prospectively evaluated differences in viremia and viruria with three immunosuppressive combination therapies. Viruria was highest with tacrolimus-MMF combination (46%) compared with cyclosporine-MMF (13%) therapy, but the choice of calcineurin inhibitor or adjuvant immunosuppression did not influence viremia or nephritis.

Shi et al. (12) showed significant higher occurrence of BKVN with the use of 15-deoxyspergualin to treat acute rejection (57.1 versus 3.7%; P < 0.001). They also found an increased risk with the combination of tacrolimus and MMF therapy (P = 0.003). Thus, risk factors such as HLA mismatches, use of tacrolimus-MMF immunosuppression, and BKV-specific immune deficiencies have been identified.

**Short- and Long-Term Grant Survival**

In the late 1990s and early 2000s, BKVN resulted in irreversible graft failure in 30 to 60% of cases. This occurred because of lack of awareness, misdiagnosis, late diagnosis, and inadvertent use of intensive immunosuppressive therapy for presumptive acute rejection (4,6).

The actuarial kidney graft survival for patients with BKVN has improved in the past decade. The 1-, 3-, and 5-yr actuarial kidney graft survival for patients with BKVN at our center (n = 58) was 94.8, 68.4, and 57.6%, respectively (62). This is substantially better than our earlier series of 89.5, 57.9, and 47.4%, respectively (67). This is due either to early detection of viremia and resolution of viremia with therapeutic intervention to prevent the occurrence of nephritis or to detection of minimal histologic changes of nephritis during routine surveillance biopsy. In recent years, transplant patients with viremia are submitted for renal biopsy, leading to diagnosis of subclinical nephritis without renal dysfunction. Thus, bias toward early diagnosis may be influencing further graft survival in recent years. Treatment strategies have shown significant short-term improvements, such as elimination of circulating viremia; however, long-term events, such as late acute and chronic rejections, need to be investigated.

Repeat transplant can be performed successfully after primary graft failure secondary to BKVN (68,69). Ramos et al. (69) reported successful repeat transplantation in nine of 10 patients without recurrent BKVN. Womer et al. (68) recently reported successful repeat transplantation in two patients despite active viremia. Transplant graft nephrectomy has been advocated and seems prudent before repeat transplantation to eliminate the graft that is infected with BKV; however, there is no evidence to support this practice. Regardless, the risk for recurrent BKVN after second transplantation is possibly real and should not be ignored.

**Conclusions**

BKV infection is an important clinical problem in kidney transplant recipients and is most likely due to the enhanced immunosuppressive state and BKV-specific immune deficiency with alloimmune activation. Using diagnostic tools such as BKV DNA in urine and or plasma and careful renal histologic evaluation are critical to making the diagnosis. More important, therapy with reduction in immunosuppression and/or antiviral therapy with careful monitoring of patients with BKVN is of paramount importance to prevent progressive renal graft failure. Screening for viremia or viruria can be used to identify early infection, and preemptive reduction in immunosuppression for patients with viremia can decrease the prevalence of BKVN. In recent years, early diagnosis, prevention, and prompt treatment of BKV infection have improved short- and long-term graft survival.

**Disclosures**

None.

**References**


