

# BK Virus–Associated Nephropathy in Kidney Transplant Recipients

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## HISTORICAL ASPECTS

BK virus was first isolated in 1970 from a kidney transplant recipient with a ureteric stricture. Epidemiologic studies have shown that up to 90% of some human populations become exposed to BK virus by adulthood. After kidney transplant, 10% to 60% of patients excrete the virus in their urine. However, viruria is typically asymptomatic or associated with only transient graft dysfunction.

A new era in the study of BK virus began when a patient was diagnosed (by needle biopsy) with a BK virus infection in 1993 and the finding was subsequently published in 1996. In the following years, additional cases were reported by kidney transplant centers worldwide.<sup>1</sup>

Polyomavirus continues to represent a formidable challenge in kidney transplantation.

BK virus–associated nephropathy is a relevant topic in the new era of transplantation. This condition has profound implications in allograft survival and quality of life. With the present shortage of available organs for transplantation, it is our mission to provide all tools accessible to us to prolong patient and allograft survival.

Before screening protocols for BK nephropathy were put into place, results were very disappointing, and the transplant community witnessed the rapid failure of allografts meant to last 10 to 20 years. Fortunately, with the development of new techniques for screening, and a better understanding of the immunobiology of the BK virus, we are witnessing a better prognosis for transplant recipients who develop BK nephritis.

## EPIDEMIOLOGIC ASPECTS

Primary infection typically occurs during early childhood, after the waning of maternal antibodies. Before the age of 10 years, the seroprevalence increases to 50% and reaches more than 70% in adults. In the 1960s, exposure to polyomavirus resulted from contaminated polyomavirus and adenovirus vaccines.

The natural route of BK virus transmission has not been resolved and may be respiratory or oral. BK virus is fairly resistant to environmental inactivation. A state of nonreplicative infections, termed *latency*, is established in renal tubular epithelial cells. Activation and low-level replication with asymptomatic viruria occurs in 5% of healthy individuals. The prevalence may increase with pregnancy or immune dysfunction to reach more than 60%. In renal transplant recipients, the prevalence rate of polyomavirus nephropathy varies from 1% to 10%, reflecting the use of different immunosuppressive protocols and diagnostic approaches.<sup>2</sup>

## MODE OF TRANSMISSION

- a. The donor kidney itself.
- b. Urine.
- c. Nasopharyngeal aspirate obtained from infants with respiratory infections.
- d. The possibility of fecal/oral transmission recently has been raised by the demonstration of viral DNA in urban sewage.
- e. Blood, semen, genital tissues, and normal skin biopsies have also been shown to contain BK virus.
- f. Transplacental transmission of polyomaviruses from mother to fetus has been recorded.<sup>1</sup>

## IMMUNITY-RELATED RISK FACTORS FOR POLYOMAVIRUS-ASSOCIATED NEPHROPATHY

- a. Intense triple-drug immunosuppression with agents including calcineurin inhibitors, T-cell depleting agents, and steroids.
- b. Renal transplant.
- c. HLA antigen mismatches.
- d. Rejection and antirejection treatment.
- e. BK virus–seronegative recipients.
- f. BK virus–seropositive donors.
- g. HLA–C7–negative donor, negative recipient.

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- h. Low number BK virus–specific interferon- $\gamma$ –producing T cells.<sup>3</sup>

## VIROLOGY

The complete genome of BK virus contains 5,153 bp. It is functionally divided into 3 regions: the early, late, and transcriptional control region (TCR). The first region codes for the small and large T antigens. The second region codes for the viral capsid proteins VP1, VP2, and VP3 and the agnoprotein. The last region (TCR) contains the transcriptional control elements for both early and late gene expression. Primary transcripts are required for viral replication. Late transcripts encode viral capsid proteins and agnoproteins that play a critical role in the regulation of viral gene expression and replication and in the modulation of certain important host cell functions, including cell cycle progression and DNA repair. It is not known if genetic alterations are essential for the pathogenesis associated with BK virus after a kidney transplant; nevertheless, BK virus strains with rearranged TCR have been specifically described in association with immunosuppressive therapies.<sup>4</sup>

## INFLUENCE OF IMMUNOSUPPRESSION ON BK VIRUS

### Type of Immunosuppressive Regimen

BK virus nephropathy has been diagnosed in patients receiving a maintenance therapy consisting of different drug classes, such as calcineurin inhibitors, antimetabolites, mammalian target of rapamycin inhibitors, and corticosteroids. Before 1995, BK virus nephropathy was rarely identified as a clinical problem in renal transplantation. New immunosuppressive agents and their use in combination therapy have merged as a causative factor in the occurrence of BK virus infection. The use of tacrolimus compared to cyclosporine, or mycophenolate mofetil (MMF) compared to azathioprine, has been implicated as a major determinant of BK viremia and BK virus nephropathy. An increased risk of BK virus replication and BK virus nephropathy with tacrolimus, MMF, and corticosteroid combinations was demonstrated in prospective and retrospective histopathology studies.

Brennan et al<sup>5</sup> prospectively evaluated the differences between viremia, viruria, and BK virus nephropathy with 3 different immunosuppressive regimens. Patients were randomly assigned to receive tacrolimus or cyclosporine, as patients given second agents routinely received azathioprine. MMF was substituted for azathioprine under certain circumstances. All patients received prednisone, which was tapered to 5 to 7.5 mg daily by month 3. By year 1, 35% of patients developed viruria and 11.5% viremia; neither were affected independently by tacrolimus, cyclosporine, azathioprine, or

MMF. The study revealed no difference in the rate of BK viruria or viremia among those receiving tacrolimus compared to cyclosporine. As well, no differences were found with azathioprine compared to MMF.

Of the 4 possible combinations of calcineurin inhibitors and antimetabolites, the cyclosporine-MMF combination was associated with the lowest incidence of viruria and viremia, and tacrolimus-MMF with the highest. This study<sup>5</sup> illustrates that BK virus infection is not specific for certain immunosuppressive agents, but tacrolimus-MMF combination is the most permissive regimen for BK virus reactivation. BK virus has also been seen occasionally in recipients receiving calcineurin inhibitor–free immunosuppressive regimens. Some studies suggest that avoidance or early cessation of steroids may be associated with a lower incidence of BK virus nephropathy.

In a retrospective single center analysis of 213 kidneys and 14 kidney/pancreas transplants,<sup>6</sup> early steroid cessation (later than 7 days), or steroid avoidance regimens, resulted in a lower incidence of BK virus nephropathy (0% versus 3.5%).

## DOSING OF IMMUNOSUPPRESSION

When looking at the influence of drug concentration and dosing and the occurrence of BK virus reactivation, higher doses of tacrolimus (trough levels of more than 8 ng/mL) or MMF have been associated with BK virus replication and BK virus nephropathy. Moreover, the reduction of tacrolimus trough levels, from more than 9 ng/mL to 6 ng/mL, and of MMF to a daily dose of less than 1 g resulted in improvement or stabilization of BK virus nephropathy in 9 of 10 cases.<sup>7</sup>

In the prospective study of Brennan et al,<sup>5</sup> the differences between BK viruria and BK virus nephropathy with 3 different immunosuppressive regimens were evaluated by using tacrolimus trough levels of 5 to 10 ng/mL. Identification of BK viremia triggered discontinuation of azathioprine or MMF. If viremia failed to clear within 4 weeks, the tacrolimus dose was tapered to levels of 3 to 5 ng/mL. One year after treatment, reduction of immunosuppression was associated with clearance of viremia in 22 of 23 patients with viremia. The mean time to clearance was 54 days. In 7 patients, viremia cleared after cessation of MMF or azathioprine alone. For 2 patients, the calcineurin inhibitor (cyclosporine or tacrolimus) dose alone was decreased.

## KIDNEY BIOPSY

Biopsy of kidney remains the “gold standard” for the diagnosis of BK virus–associated nephropathy. In addition, biopsy evaluation provides irreplaceable means of assessing the extent of tissue damage and disease progression, as well as the degree of associated inflammatory response and scarring.<sup>8</sup>

The characteristic findings on light microscopy are intranuclear basophilic viral inclusions in epithelial cells of the urothelium. These are found in the medulla or cortex and are multifocal with random distribution.

In early disease (pattern A), the cytopathic changes are present with little to no inflammation or tubular atrophy.

Pattern B consists of viral cytopathic changes with varying degrees of inflammation, tubular atrophy, and fibrosis.

In late BK nephropathy (pattern C), cytopathic changes often are less apparent, as a result of a background of tubular atrophy, interstitial fibrosis, and chronic inflammatory infiltrate. Other changes described include glomerular crescents, ischemic glomerulopathy, transplant glomerulopathy, abundant plasma cell infiltrates, and tubular microcalcifications.

It is not uncommon to see changes that mimic acute cellular rejection with lymphocytic infiltrates. In these cases, the immunostain for simian virus 40 is invaluable for differentiating BK nephropathy (positive immunostaining) from acute cellular rejection (negative immunostaining). The presence of positive C4d staining or endothelialitis indicates probable acute cellular rejection with humoral component.<sup>9</sup>

Even a kidney biopsy has limitations owing to the focal nature of this condition. It is advisable to evaluate 2 or more cores to reduce sampling errors. This problem can occur in up to 36% of cases if only 1 tissue core is evaluated. Some authorities also recommend obtaining medulla to increase the sensitivity of the biopsy specimen.

The potential clinical implications of differentiating rejection and BK virus-associated nephropathy are clear. The treatment for rejection requires the use of more aggressive immunosuppression, whereas the treatment for BK virus-associated nephropathy is reduction of immunosuppression.<sup>10</sup>

## **BK VIRUS POLYMERASE CHAIN REACTION VERSUS CYTOLOGY**

Viruria can be monitored by cytology or by quantification of viral DNA. Virus-infected cells (“decoy cells”) found in urine samples are characterized by large homogeneous basophilic nuclear inclusions, which may mimic the nuclear changes in urothelium cancer.<sup>11</sup> Accurate interpretation of urine cytology requires a trained pathologist for distinguishing among viral infection, reactive atypia, and urothelial dysplasia.

By morphologic features alone, one cannot always distinguish between BK virus and other viral infections. Decoy cells may be a result of infection with BK virus, JC virus, and less commonly, adenoviruses. BK virus polymerase chain reaction (PCR) may ultimately

prove superior for the screening of polyomavirus-associated nephropathy.

One study<sup>12</sup> compared BK virus PCR with urine cytology by evaluating 114 patients for evidence of BK viruria by PCR and cytology alone, with concurrent renal biopsy to correlate with actual polyomavirus-associated nephropathy. Results indicated that cytologic identification of decoy cells as a marker of polyomavirus by viruria had a sensitivity of 25% and a specificity of 84%, whereas BK virus PCR in urine for BK viruria had a sensitivity of 100% and a specificity of 78% for concurrent polyomavirus-associated nephropathy. BK virus PCR in plasma for BK viremia was even better, with a sensitivity of 100% and a specificity of 91%. Also, PCR test of BK virus in plasma and urine had superior positive and negative predictive values for biopsy-proven, concurrent polyomavirus-associated nephropathy.

The interval from the onset of viruria to onset of viremia is 1 to 3 months. Furthermore, viremia precedes BK virus nephropathy by a median of 1 to 12 weeks. In response to reduction of immunosuppression, viremia resolves before viruria. Knowledge of this viruria-viremia-nephritis sequence provides a basis for generating algorithms for testing urine and blood specimens in renal transplant recipients.<sup>13</sup>

The positive predictive value of viremia for BK virus nephropathy is around 60%, which is higher than for viruria. Since viruria is more sensitive than viremia, some institutions (including ours) use a BK virus screening strategy based primarily on urinary surveillance by real-time quantitative PCR, with monitoring of plasma only if the urine viral load is positive or rises above a predetermined threshold level that correlates with high specificity for BK virus nephropathy. This strategy is likely more cost-effective than monitoring both urine and plasma.<sup>2,13,14</sup>

## **VIRURIA AND VIREMIA FOR BK VIRUS NEPHROPATHY**

To facilitate clinical management of renal transplant recipients, it would be desirable to identify relevant threshold values of BK viruria and BK viremia for a presumed diagnosis of BK virus nephropathy. In general, renal transplant recipients with BK virus nephropathy have a higher BK viral load than transplant recipients without BK virus nephropathy; however, there are no clear threshold levels for urinary viral loads that could predict viremia, and no universal viruria and viremia cutoff values exist for BK virus nephropathy. Threshold values yielding a specificity of 93% or more for BK virus nephropathy had been proposed. Some studies report using the following threshold values: plasma BK viral load, 10,000 copies or more per milliliter; VP1 mRNA load,  $6.5 \times 10^5$

copies/ng total RNA. Hirsch et al<sup>15</sup> suggest that the presence of polyomavirus loads persisting for more than 3 weeks above these thresholds is highly suggestive of BK virus nephropathy (“presumptive polyomavirus-associated nephropathy”).

## TREATMENT OF POLYOMAVIRUS-ASSOCIATED NEPHROPATHY

Reduction or adjustment in immunosuppression remains the cornerstone for the treatment and prevention of polyomavirus-associated nephropathy. Because the reconstitution of the immune system in the control of infection takes 4 to 12 weeks, it is imperative to start treatment as early as possible. The one risk encountered with reduction in immunosuppression is the development of acute rejection. The latter is uncommon (10%–15%) particularly in patients diagnosed early.

The preliminary results of Wali et al<sup>16</sup> reflect the protocol used at the University of Maryland. This protocol has resulted in clearance of viremia with no graft loss or significant rejection diagnosed. Specifically, the immunosuppression reduction strategy is as follows: step 1, decrease in the dose of MMF by 50% immediately after diagnosis; step 2, 50% decrease in the target trough level of tacrolimus at 3 months if decoy cells persist; and step 3, elimination of MMF at 6 months if decoy cells persist. In our center, we usually discontinue the antiproliferative agent (MMF) with subsequent monitoring of the viral load every 2 weeks. If there is no response, we reduce the calcineurin inhibitor, targeting a tacrolimus level between 3 to 5 ng/mL or a cyclosporine level between 50 and 100 ng/mL. In general, with a dual therapy (tacrolimus and prednisone), or more recently with a single agent (tacrolimus), we have observed excellent response, with stable glomerular filtration rates and prolonged graft survival.<sup>16</sup>

In addition to the decrease in immunosuppression, several centers have reported the use of several anti-polyoma viral agents with anti-BK virus in vitro activity. These include cidofovir, leflunomide, quinolones, and intravenous immunoglobulin. The efficacy of these antiviral agents is difficult to determine, as they have been used in combination with other drugs—with reduction in immunosuppression—and even, in certain cases, in combination with each other. In addition, no prospective randomized control trials have been conducted.<sup>17</sup>

In summary, examination of the available literature demonstrates that none of the ancillary treatments have been conclusively proven to be efficacious. Most studies were neither randomized nor double-blinded, and histologic grading of polyomavirus-associated nephropathy was often missing. Multicenter prospective studies are needed to clarify this important issue by stratifying histologic grading, renal function, viral load diagnosis, and most importantly, evaluating different strategies,

assessed independently. Early diagnosis with close monitoring of renal function and serial determinations of viremia and viruria continue to represent the most efficacious tool to control polyomavirus-associated nephropathy. Systemic reduction in immunosuppression has not been associated with clear evidence of increased chronic rejection, but longer times of follow-up and more stringent studies are necessary to determine the long-term impact of the interventions for polyomavirus-associated nephropathy on long-term graft outcomes.

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