Review article

BK virus: microbiology, epidemiology, pathogenesis, clinical manifestations and treatment

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Background: BK virus infection is common but is usually asymptomatic. However, it can become life threatening as severe hemorrhagic cystitis (HC) or the polyomavirus-associated nephropathy (PVAN) particularly in immune compromised and transplant recipients. Some investigators have studied the pathophysiology and there are anecdotal and uncontrolled studies of therapy with few conclusions allowing treatment guidelines.

Objectives: Summarize literature review of current knowledge concerning the nature, epidemiology, pathophysiology, diagnosis and treatment of this common virus infection.

Results: HC is a not uncommon and often misdiagnosed infection from BK virus. It is usually self limited but can become life threatening in immune compromised patients. PVAN threatens survival of transplanted kidneys and is difficult to differentiate from rejection without sophisticated molecular diagnostic technology. We have sufficient information for making a diagnosis of BK virus disease by using clinical, serological and molecular technology. Studies using manipulation of immunosuppression and a variety of antiviral agents, including cidofovir, leflunomide, intravenous immunoglobulin, vidarabine, fluoroquinolones, have been published but most were uncontrolled reports of few cases. Cidofovir offers some promise but more must be learned before there is hope for evidence-based treatment guidelines.

Keywords: BK-virus, clinical feature, diagnosis, epidemiology, pathophysiology, polyoma virus, treatment

Currently, two major diseases associated with BK virus have been recognized in the past decade, namely, Polyomavirus-associated nephropathy (PVAN) in solid organ transplants (especially kidney) and hemorrhagic cystitis (HC) in allogeneic hematopoietic stem cell transplantation (HSCT) recipients. Better understanding of the pathophysiology of the BK virus coupled with newer diagnostic tools has increased recognition of these potentially devastating diseases.

BK virus belongs to the Papovaviridae family which includes two genera, the papilloma and polyomaviruses. Two human pathogens, JC and BK viruses are both polyomaviruses. Both are nonenveloped viruses with 72 capsomere icosahedral capsids and a double-stranded supercoiled loop of DNA. These small (42-45 nm) viruses weigh 3.4x10^-6 grams and have about 5000 base-pairs.

Functionally, the genome of BK consists of three main components.

1) An early region codes for large T and small t antigens. These antigens accumulate in the nucleus, stimulate cellular growth and are important in replication. Early proteins are associated with immortalization and transformation. A large-T antigen binds to tumor suppressor proteins Rb and p53 and prevents cell death [1].

2) A late region codes for viral capsid proteins VP1-3 and agnoprotein which are required for cell entry and virus assembly. Agnoprotein is thought to be involved in promoting release of the completed virion from the cell.

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3) A noncoding control region (NCCR) has a high degree of sequence variability and might contribute to the degree of virulence. It is the origin of replication and transcription. It also contains control region promoters and enhancers which are involved in regulation of both early and late gene transcription [2].

The growth cycle is 36-44 hours. In the immunocompromised host, the virus can multiply exclusively in the nucleus resulting eventually in cell death.

BK strains are genotypically classified based on polymorphisms in the VP1 region (genotypes I-VI), and the NCCR structure [3]. BKV variants are classified as either archetype or rearranged forms. BK variants with rearranged NCCRs are not considered as unique strains. The archtypical BK NCCR structure is the most common form found in urine.

**History of discovery**

Only two Polyomaviruses are known to infect humans, JC (polyomavirus hominis type 1), and BK (polyomavirus hominis type 2). These two viruses are closely related genetically (sharing 74% of their genome sequence). BK was named after the initials of a Sudanese patient in whom the virus was first isolated in England in 1970 as part of the central public health laboratories created to battle infections during the 2nd World War. Dr. Sylvia Gardner, a virologist recognized an infected cell after the patient, who was receiving azathioprine and prednisolone following a kidney transplant, presented with a ureteral stricture and urinary shedding of inclusion-bearing epithelial cells five months after transplantation. The viral particles resembled a papilloma virus. Subsequently, the unique viral cytopathic effects distinguished this virus from papilloma virus after inoculation of the urine specimen to cell culture. The clinical syndrome of Polyomavirus-associated nephropathy (PVAN) was described decades after the discovery of the virus itself. In 1995, a patient in the USA developed nephropathy following renal transplantation. Histopathology of the kidney demonstrated focal tubulitis, and viral inclusion bodies confirmed BK by immunohistochemistry [4]. A decade earlier, in the mid 1980’s, a patient with BK virus induced HC following bone marrow (BM) transplantation was described [5].

In contrast, JC was discovered much earlier than BK. A rare entity, progressive multifocal encephalopathy (PML) was described in 1958. The causative pathogen JC virus was detected by electron microscopy (EM) in 1965 and then isolated from the brain of a patient with Hodgkin’s disease, the same year (1970) BK was discovered [6].

JC and BK viruses can be isolated from kidney. JC alone can be isolated from the brain in cases of PML. SV40, a polyomavirus in animals, may cause a PML-like clinical picture in rhesus monkeys. Both BK and JC may cause malignant transformation through inhibition of the gene p53 in animal models but not in humans [7]. Polyoma came from “(-poly) many (-oma) tumors” from the Greek language. The clinical syndrome of BK includes viruria, viremia, ureteral ulceration/obstruction and HC.

**Epidemiology**

BK type I has a higher prevalence between the two types. Serologic studies have demonstrated that the incidence of BK antibodies are independent of gender, socioeconomic status, and rural versus urban residence [8]. Maternal antibodies to BK can be detected in neonates during the first few months of life. In several early reports, 60-100% of children have antibodies to BK by age 10. These antibodies then decline to around 70% in older persons. Worldwide, BK virus infection is ubiquitous with a seroprevalence of 75% (range 46-94%) in adults [8]. The route of transmission is presumed to be human-to-human as there is no animal reservoir identified. Body fluids, which may transmit infections, include sputum, urine, semen, and feces, as well as donated organs.

Risk factors for transient asymptomatic reactivation of BK virus include pregnancy (especially third trimester), older age, diabetes, cirrhosis, SLE, and anti-neoplastic chemotherapy. Up to 5% of normal hosts have asymptomatic viruria. In pregnancy, viruria develops in 10-47%, especially during the second and third trimesters. Shedding continues intermittently until after delivery. A rising antibody titer to BK has been noted in 14% of pregnant women [9].

**Clinical features/pathogenesis**

Polyoma virus clinical manifestations were categorized by Hirsch (Table 1), based on viral latency and prevalence [10].

Polyomavirus-associated nephropathy (PVAN), BK virus-allograft nephropathy (BKVAN), BK virus nephropathy (BKN), and polyomavirus nephropathy (PVN) all describe an infectious process caused by BK occurring after renal transplantation. Laboratory
and clinical findings are characterized by viral inclusions, interstitial nephritis, and progressive allograft failure. BK most commonly causes PVAN and is strongly associated with HC in BM transplant recipients. In contrast, the JC is typically associated with central nervous system (CNS) disease and very rarely causes nephropathy. Since the term PVAN is more accepted, PVAN will be used herein.

Serological studies suggest that BK infection is common, but primary infection is rarely identified. It is usually asymptomatic and occurs during childhood. It arises through the respiratory tract leading to mild respiratory symptoms or tonsillitis in both immunocompetent and immunocompromised hosts [11]. Immunosuppressed children may present with cystitis or nephritis. Unusual manifestations of primary infection include encephalitis and the Guillain-Barré syndrome [12].

The virus multiplies in the respiratory tract, and then spreads to other organs though the blood stream. BK virus remains clinically silent, in the kidneys, in immunocompetent hosts. Subsequently, it may be activated during immunosuppression. The lymphocytes are a secondary site of viral latency. As with the latency of herpes virus, BK virus remains within a cell in a non-replicating or minimally replicating state. The viral genome may remain either episomal or integrated in the host genome. In this way, the virus escapes immunosurveillance. In addition, sporadic BK virus reactivation may occur in patients with congenital immunodeficiency or secondary immunodeficiency such as HIV [13].

Both BK virus and JC virus can be identified in the latent or reactivated state in the kidney, but only JC virus infects the CNS, causing PML. Latent BK virus can be found mainly in urogenital organs including 50% of native kidneys and 40% of ureters. Other organs harboring foci of latent infection include the heart, lungs, tonsils, spleen, and lymph nodes. Those account for latent infection patients who later developed PVAN, even after they have undergone pre-transplant bilateral native nephrectomy.

Impairment of cell-mediated immune function has been associated with reactivation which begins with active viral replication in the graft, followed by viral shedding in the urine and finally, viremia. Tubular epithelial cells are common targets. The virus multiplies exclusively in the nucleus resulting in cell death. However, Leung et al. has demonstrated no correlation between viruria and viremia. They postulate that the independent reactivation of renal and extrarenal sources of latent BK virus must occur independently [14]. Removal of the renal nidus of the virus allows clearance of the viremia. Moreover, the pathogenesis of BK virus reactivation is thought to be based on hormonal and immunologic changes, which may occur in the elderly and during pregnancy. Reactivation of BKV in the elderly, pregnant women or their fetuses does not produce clinical disease.

BK-induced HC rarely follows solid organ transplantation. It is much more frequent following allogeneic HSCT (5-60%). Conversely, renal involvement is rare after allogeneic HCT, but common among kidney transplant recipients. Previously damaged tissue by surgery and cold ischemia may promote BK replication, explaining why infection in renal transplant recipients is generally a tubulointerstitial nephritis.

In the immunocompromised host, using anti-lymphocyte globulin for induction therapy is less likely to cause reactivation of BK when it is used to prevent rejection. The latter, with its stronger immunosuppressive effects is associated with polyoma virus replication [15]. Interestingly, reactivation of BK is generally not associated with reactivation of CMV.

Risk factors for BK virus infection (Table 2)

a) The degree of HLA mismatch

b) The type of immunosuppressive therapy to prevent rejection or graft-versus-host disease.

In addition to immune dysfunction, immunosuppressive
therapy may potentate polyomavirus infection by facilitating the development of virulent mutant strains [16].

c) Tissue injury/cellular regeneration. This could explain why organs other than the transplanted organ, under the same degree of immune-suppression, are not affected by BK virus [15].

d) Seronegativity promotes prolonged viremia and progression to PVAN.

e) HIV regulatory protein (tat) up-regulates JC virus transcription possibly explaining the high frequency of BK virus in HIV patients [17].

The oncogenicity of BK virus is a matter of controversy, notably, the prevalence of BK virus is quite high in the cancer-free population. However one case report described bladder cancer developing in a renal transplant recipient following BK infection [18].

Tubulointerstitial nephritis in a patient with primary immunodeficiency and cystitis in healthy children have also been reported [19].

Clinical syndromes based on host factors

Immunocompetent host

Serological studies suggest that infection is common in humans, but primary polyomavirus infection is rarely identified and poorly characterized. Primary infection is usually asymptomatic and occurs during childhood through the respiratory tract via aerosol or contact with fomites. Mild respiratory symptoms or tonsillitis may follow in either immunocompetent or immunocompromised hosts [11]. Children may present with cystitis or nephrotic syndrome. Unusual presentations include encephalitis and Guillain-Barré syndrome. BK virus DNA was detected in 3.8% of adults with suspected encephalitis and 1.5% of pediatric cases [20].

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<td>I</td>
<td>Microscopic hematuria/dysuria</td>
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<td>Macroscopic hematuria</td>
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<td>Microscopic hematuria with clot and urinary obstruction</td>
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<td>IV</td>
<td>Gross hematuria with clots causing obstruction</td>
<td>Microscopic hematuria with clots and urinary obstruction</td>
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Solid-organ transplant recipients
Renal transplantation

Early investigations of BK and JC viruses in recipients of renal allografts showed no significant effects on graft survival and were dismissed at the time [21]. In the past decade, however, several authors have reported severe adverse outcomes including a lower rate of graft survival and irreversible worsening of serum creatinine due to BK reactivation [22, 23]. PVAN has emerged as one of the leading causes of renal allograft failure during the first two years after transplantation [24], and nowadays occurs 10-fold more than CMV infection. In contrast, only few cases of nephritis have been attributed to JC infection.

PVAN was not recognized during the use of immunosuppressive therapy such as cyclosporine A and azathioprine [25]. After the third generation immunosuppressive drugs, such as tacrolimus and mycophenolate mofetil (MMF) became available, the odds of developing PVAN increased by 13-fold [26]. The combination of tacrolimus and MMF may lead to much greater BK viral replication [15, 27]. In renal allograft recipients, exposure to tacrolimus increases the risk of PVAN when compared to cyclosporine A, even when subtherapeutic doses of tacrolimus (<8ng/mL) are used [28]. One prospective study found that up to 8% of renal transplant patients who received tacrolimus-azathioprine-prednisone or cyclosporine A-mycophenolate mofetil-prednisone developed PVAN [28]. However, less than 10% of cases of PVAN may still develop in patients unexposed to tacrolimus or MMF [28, 29].

Because seropositivity itself is not protective for disease progression, infection could represent either reactivation or primary infection in a serological mismatch. Although 10 to 45% of renal transplant...
Recipients have documented BK viruria, less than 5% develop PVAN [30]. Histologically, PVAN is characterized by tubular damage and interstitial inflammation that may progress to nephropathy and graft loss [31]. Without intervention, 45% of these renal allografts will fail [25].

BK virus localizes in epithelial cells of the renal medulla. When the dormant virus starts replicating, it progresses to the renal cortex and causes a characteristic injury to the proximal tubules. Viral particles released by cell necrosis enter the new tubular cells by endocytosis and proceed into the nucleus. Serum creatinine becomes elevated only after replication has extended to the cortex and proximal tubules. Allograft dysfunction is indicated by leakage from injured tubules. After prolonged necrosis of the proximal tubules, dysfunction is irreversible [25, 32]. Of note, the glomerular capillary is not usually involved by BK virus. This may explain why hematuria and proteinuria as clinical manifestations of infection are rare.

The clinical presentation is characterized by a transient decrease in graft function, which could be wrongly interpreted as graft rejection resulting in unnecessary use of additional immunosuppression. In addition to an elevation of the serum creatinine level, hematuria, dysuria, ureteral obstruction, and proteinuria may be part of the disease presentation. Fever is usually absent.

A discrepancy between viral reactivation and the incidence of PVAN may be ascribed to such confounding factors as the allogenic immune microenvironment within the renal allograft and genomic heterogeneity of noncoding control region NCCR [33]. Although most infections with BK appear to arise from reactivation within the urinary tract, disseminated infection with multiorgan failure has been reported following cadaveric renal transplantation [34]. Recent graft rejection with incomplete response to anti-rejection therapy should raise the suspicion of PVAN especially when late rejection (6-12 months following transplantation) is suspected. The probability of developing PVAN is less when using live and related donors than with cadaveric [25].

Clinical presentation
1. Tubulointerstitial nephritis (classic PVAN). Tubulointerstitial nephritis is the most common type of BK virus disease in renal transplant recipients. The clinical presentation may include nephropathy, hematuria, lymphoceles or obstructive uropathy. The majority of PVAN occurs in the first year (range 8-270 days), but could occur as early as 8 weeks post-transplantation [10].

2. Ureteral stenosis or stricture may develop months to years after transplantation. Viral particles are identified within proliferating ureteric epithelial cells [21]. The incidence of ureter stenosis was noted in <10% of episodes of allograft dysfunction [27]. Other postulated causes of ureteric stenosis include tissue injury by surgical procedures, acute rejection, and ischemia. CMV is a more frequent cause of ureteric pathology than BK virus [35]. In the 1970’s, pre-cyclosporine era, BK virus induced ureteric stenosis was reported more frequently than PVAN [36].

3. Unusual histologic manifestations of BK virus infection include bladder cancer, renal pelvis adenocarcinoma of the graft, lymphoproliferative disease, and disseminated vasculopathy [18, 34]. Simultaneous PVAN and Hemorrhagic cystitis is seldom reported [37].

Non-renal solid-organ transplantation
A few case reports of BK virus infections have been documented following non-renal solid-organ transplantation. The low probability of developing PVAN suggests that graft ischemia and alloimmune responses may contribute to BK reactivation. One case of nephropathy in native kidneys was described following pancreas transplantation. Unexplained nephropathy was noted nine months after transplant and subsequent urine studies revealed decoy cells and BK viruria, later confirmed by kidney biopsy. The combination of diabetic nephropathy and immunosuppressive therapy may have contributed to the infection. BK viremia, viruria, and decoy cells in the urine were reported following heart transplantation without any clinical significance or proven kidney involvement [38]. Asymptomatic viremia was reported in one liver transplant recipient [39].

HSCT recipients
In HSCT recipients, viral induced HC is the primary concern rather than nephropathy. Initially, adenovirus was recognized before BK as the principle pathogen causing HSCT-related HC [40]. More recent reports suggest that adenovirus contributes to only a minor proportion of HC compared to BK [41]. BK virus related illness is far more common than JC virus induced PML in HSCT recipients.
Conditioning chemotherapy regimens cause intense immunosuppression and inflammation of bladder mucosa resulting in activation of latent BK virus. When the viral load exceeds a certain level, the cytopathic effects of BK virus result in hematuria. Further cellular damage results from the intense immunologic response that is seen in some cases. An immune reconstitution process is suggested to be involved in the pathogenesis of BK virus HC. The abundant viral antigens are targeted by a recovering immune system during the post-engraftment period, which usually occurs at about the same time. BK virus replicates actively in uroepithelium during the pre-engraft period, only to be attacked by the recovered immune system, which in turn causes extensive inflammation, and in severe cases, bleeding [42].

Graft-versus-host disease (GVHD), a form of alloimmune reaction, is thought to be part of the pathogenesis and contributes to the high incidence of BK virus disease in allogeneic HSCT patients with documented high-grade GVHD [43]. The proposed association of BK virus disease and an active immune response in the pathogenesis of HC is not without limitations. A BK virus induced HC in a patient with lymphopenia is inconsistent with the immune reconstitution theory [44] while the use of high-dose corticosteroids or other immunosuppressive therapies is conflicting with the GVHD theory [45].

Incidence of HC, after allogeneic HSCT, varies from 10-60% due to different diagnostic criteria. Following HSCT, HC occurs four times as often in patients with BK viruria than those without it. BK virus induced HC typically appears 1-18 weeks after transplantation. The duration of viral shedding varies from 1-119 days [5]. The variability of onset and duration of re-activation is due to contributing factors such as GVHD, and degree of immunosuppression. The incidence of disease reactivation is higher in allogeneic-HSCT recipients (50%) than in autologous-HSCT recipients (7%). Prolonged disease is observed in half of allogeneic-HSCT patients but only 6.8% of autologous-HSCT recipients [5]. In other reports, however, no such differences were evident between allogenic and autologous HSCT recipients (46-53% vs. 39-57%, respectively) [46, 47].

Patients may present with asymptomatic hematuria, cystitis, HC, ureteral stenosis, and rarely interstitial nephritis (PVAN) [28]. HC is the common presentation and symptoms are similar to a bacterial urinary tract infection. Inflammation of the bladder epithelium causes frequency, urgency, spastic bladder pain, dysuria, or hematuria. Two similar scales for grading the severity of HC have been proposed [48, 49] (Table 3). In both scales, the higher grades are associated with a higher morbidity and mortality and are more likely to be caused by BK virus. In addition, duration of HC is strongly associated with > seven days of BK viruria [5]. The main contributors to morbidity and mortality due to BK virus infections are multiple blood transfusions, concurrent urinary tract infection, and secondary obstructive nephropathy. Most cases of HC will resolve spontaneously.

<table>
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<tr>
<th>Table 3. Risk factors for BKV reactivation.</th>
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<td>1. Major HLA mismatches [5]</td>
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<td>2. Immunosuppressive therapy and intensity [15]</td>
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<td>3. Tissue injury/acute rejection [74]</td>
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<td>4. Seronegativity of pediatric recipient [95]</td>
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<td>5. Viral determinant ion (genotype) [96]</td>
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<td>6. HIV infection</td>
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<td>7. Male gender [27, 97]</td>
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<td>8. Older age [98]</td>
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<td>9. High BKV IgG titer pretransplantation [62]</td>
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<td>10. Concurrent CMV reactivation [99]</td>
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<td>11. Diabetes mellitus [100]</td>
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<td>12. Use of a ureteral stent [74]</td>
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In contrast to adenovirus reactivation, BK virus reactivation does not always lead to HC. The contributory factors are GVHD, immunosuppressive therapy, prior radiation therapy, older age, the specific BK virus strain, and the degree of latent infection prior to transplantation. In most HSCT recipients, hematuria is due to prior chemotherapy, a urinary catheter device, or thrombocytopenia. Regardless of the chemotherapeutic regimen, 50% of HSCT recipients excrete polyomavirus in the urine [28]. Thus, BK viruria and hematuria may only be coincidental. However, HC lasting longer than seven days is strongly associated with BK virus reactivation [5]. Liver or renal failure and interstitial pneumonia from BK virus infection have been reported but are rare. Direct renal involvement is limited to obstructive uropathy usually from blood clotting within the bladder.

**HIV infection**

PML is a well-known consequence of polyoma infection in HIV infected patients. Incidence of BKV reactivation without histopathological changes is common in HIV patients, 10-37% [50]. Interestingly,
the frequent finding of BK virus in the urine was significantly higher in HIV patients than in transplant recipients in one study [51].

Active disease caused by BK virus is rare in HIV patients. To date, only BK virus induced PVAN, disseminated pulmonary, and CNS infection have been reported [52-55]. The incidence of BK viral shedding is higher when CD4 counts are lower [56]. There is an association between CD4 count < 50 cells/μL and renal failure, even in the absence of other contributing factors [28]. Atypical presentations of BK virus infection include meningoencephalitis, pneumonitis, and retinitis. These variant presentations have been seen more frequently than PVAN or HC in the setting of advanced HIV infection [54].

Systemic lupus erythematosus (SLE)

The presence of BK virus has been reported in 16-65% of patients with SLE independent of immunosuppressive treatment [57]. Viral histone/large T antigen complexes are thought to trigger autoimmune responses including anti-DNA antibodies, the histone antibodies commonly found in SLE.

Laboratory diagnosis

Overview

Primary polyoma infection of the respiratory tract is clinically insignificant. Because BK virus is rarely recovered from respiratory secretions, a viral swab is not indicated. Anti-BK IgM indicates recent infection or reactivation but may also persist for years following infection. Reactivation is accompanied by rising and persistently elevated antibody titer.

Hemorrhagic cystitis

Shedding of inclusion-bearing decoy cells from the superficial transitional layer of the renal pelvis and ureters can be identified by Papanicolaou stain. Decoy cells show characteristic viral cytopathic effects compatible with the presence of BK virus. These effects consist of cells with enlarged rounded nuclei that manifest a ground- or etched-glass appearance on the nucleoplasm. The chromatin is marginalized and has clearings. There are no intranuclear basophilic inclusions with artefactual halos as can be seen with human cytomegalovirus, which also has cytoplasmic inclusions. The name “decoy cell” is used to prevent confusion with descriptions of the nuclear changes of malignancy. Despite these discrete morphologic findings, cytological methods are not highly sensitive and viral excretion may occur in the absence of decoy cells. Cytological morphologic changes from JC and BK virus may be distinguished from each other by techniques such as immunofluorescence staining or EM [8]. Routine use of culture from urine or other body fluids for isolation of BK virus or JC virus cannot be recommended. These are particular agents that show tropism for selected cell lines, most of which are not commercially available but only in research settings. Primary human fetal glial cells must be used for isolation of JC virus. BK virus has a wider host cell range since human embryonic kidney cells as well as primary human fetal glial cells can be used. Rapid detection using shell vial assays has been described [58].

EM for viral particles in urine requires at least 10^6 viral particles/mL for diagnosis. However, EM is not practical for routine use due to cost, and labor-intensity. It is more useful as a research tool allowing detection of viruses of multiple etiologies.

The approach to the diagnosis of hemorrhagic cystitis

Microscopic hematuria is commonly seen in HSCT recipients. Determining the cause of hematuria in these patients can be challenging given the wide differential of etiologies, including thrombocytopenia, trauma from urinary catheter devices, medications, or infection. Mild HC (grade I) usually resolves spontaneously. Severity of >grade II or any degree of persistent hematuria is an indication for further evaluation.

Decoy cells

Decoy cell screening is useful for early diagnosis of BK virus induced HC with a sensitivity of almost 100%, but a low positive predictive value (<30%), since half of disease-free HSCT recipients excrete BKV at some point during the post-transplantation period [25]. Because the proportion of infected cells is highly variable, this technique has been replaced with the molecular biology method.

Molecular diagnostics

Urine. BK virus excretion in the urine usually begins within two months following HSCT transplantation in 37-49% of patients. Half of these patients do not develop HC. BK virus DNA of more than 10^6 copies/mL found in a urine sample is associated with HC [59]. Higher levels of viruria provide both positive and negative predictive value for the risk of
developing HC. It is more severity than in the absence of viruria [60]. Large quantities of BK viral DNA in urine are commonly found with HC, and not usually in asymptomatic transplant recipients or immunocompetent patients [46]. Duration of viruria varies from few weeks to several months. Most HSCT recipients with viruria are asymptomatic but have a significantly higher incidence of microscopic hematuria. The onset and termination of viruria often coincides with resolution of HC. When symptoms of HC are present, BK viruria can be demonstrated in as many as 56-80% of patients [61]. HC is linked to BK virus reactivation when a peak urine viral load of $10^9$ copies/mL is reached or if there is an increase $\geq 3$ log over baseline [62].

Recurrent viruria is a strong disease predictor. One study found that $> 90\%$ of patients with only a single episode of BK viruria were asymptomatic [47]. Viruria is most likely to be detected prior to and during an episode of HC.

**Blood.** The positive predictive value of BK viremia is greater than viruria [63]. Viremia is usually accompanied by viruria. With excellent sensitivity for BK viremia, a negative predictive value of 100% has been reported [64]. Erard et al. described a high correlation of viremia with HC when plasma BK DNA levels exceeded more than 10,000 copies/mL [44]. Others however have failed to find a consistent relationship between the quantification of viremia and HC [65]. Detection of VP-1 mRNA is an alternative to BK DNA PCR.

Drug or radiation-induced cystitis should also be included in the differential diagnosis of HC. Typically, cyclophosphamide, busulfan, or ifosfamide-related HC may occur within one week following transplantation. Acrolein, a metabolite of cyclophosphamide and ifosfamide, is toxic to bladder epithelium in an animal model and usually causes inflammation, ulceration, hemorrhage, and necrosis within few hours of administration [66]. In humans, BK HC usually occurs 10 days following HSCT, lasts longer than a week, and is accompanied by persistent BK viruria. Given the widespread use of mesna and forced diuresis to minimize chemotoxicity, Bedi et al. proposed that most cases of HC following HSCT, especially in the absence of aforementioned antineoplastic therapies, are related to BK infection [47]. Other possible viral etiologies of HC include CMV and adenovirus. The significance of BK viremia in a non-organ transplant population is unknown.

EM can reveal intracellular and extracellular viral particles but cannot distinguish between JC and BK viruses [67]. Nevertheless, the sensitivity of EM for detecting BK is inferior to PCR. Serological testing has a limited clinical role because it mostly indicates prior exposure to BK. Histopathology plays a limited role in diagnosing BK HC, unlike PVAN where it is the preferred method of diagnosis.

In summary, when BKV HC is suspected, BK DNA from both blood and urine should be obtained in addition to ruling out other possibilities of HC. If symptoms persist, accompanied by an increasing BK DNA, of urine and especially serum treatment should be considered.

**Polyoma virus associated nephropathy**

**Histopathology**

Diagnosing PVAN rests on histopathological examination and immunohistochemical confirmation [15, 23, 68]. Besides viral cytopathic effects, a focal invasion of tubules with tubulitis is characteristic of BK infection. Cytopathic changes include tubular injury with cellular enlargement, marked nuclear atypia, epithelial necrosis, focal intratubular neutrophilic infiltration, viral inclusion bodies, and mononuclear interstitial infiltration. BK virus inclusion body is described as a well-defined mass of basophilic or amphophilic homogenous intranuclear material with peripheralization of nuclear chromatin. These inclusions in the proximal and distal tubules support BK infection. The affected nuclei may become aggregated and are easily recognized at low magnification. Inflammatory cell infiltrates and tubulitis in biopsies with PVAN may represent an immune response to the infection or concurrent allograft rejection, and it is imperative to distinguish the two entities. A definitive diagnosis of rejection concurrent with viral nephropathy should only be made if there is endarteritis, fibrinoid arterial necrosis, glomerulitis, or accumulation of the complement degradation product C4d along peritubular capillaries. Mononuclear infiltrates are prominent in acute rejection, while neutrophils, and plasma cells suggest PVAN.

The degree of cytopathic changes, inflammation and fibrosis have been semi-quantitatively assessed and classified into three histopathological stages:

**(Pattern A)** cytopathic changes of tubulointerstitial cells without renal function impairment and without inflammation.
(Pattern B) Inflammation without fibrosis. Extensive renal involvement with inflammatory cell infiltrates causing cell atypia and necrosis. This stage must be distinguished from acute organ rejection. (b1 less than 25% involvement, b2 25-50% involvement, b3 involvement >50%)

(Pattern C) irreversible interstitial fibrosis and scarring.

A lower histological score significantly correlates with graft prognosis and clearance of BK virus infection. Stage B and C are significantly associated with graft loss as well as a mean serum creatinine of 3 or above at the time of renal biopsy [69].

Demonstration of major histocompatibility (MHC) class II molecules in tubular epithelium (HLA-DR) and complement degradation products, C4d, are well-established immunohistochemical markers of acute rejection. These help to distinguish acute rejection from BK infection. A large proportion of CD20 cells staining positive in renal histology are more consistent with PV AN than the natural killer or cytotoxic T cells found in acute graft rejection [70]. Graft rejection concurrent with PV AN has been reported [25].

While pathologic findings of JC virus/BK virus, CMV, HSV, and adenovirus are distinct, immunohistochemistry stains or EM may be helpful in early stage disease when cytopathologic effects are not yet obvious [71].

Urine cytology

Detection of decoy cells is a good screening test due to its highly negative predictive value. However, the positive predictive value for nephritis is low (<30%) Decoy cells can be detected in 12% of renal transplant recipients. Decoy cells may originate anywhere along the urogenital tract besides renal tubule. It is unclear if the proportion of decoy cells in the urine is related to the probability of PVAN. One particular study revealed that > five decoy cells per 10x high power field have a negative predictive value of 100% [25]. Decoy cells may be seen 1-18 months before diagnosing PVAN.

Molecular diagnostic techniques

Urine. Laboratory monitoring strategies for BKV are still evolving. Quantitative nucleic acid-based viral load assay of urine or blood are becoming widely used for BK screening. Either urinary viral DNA load or messenger RNA VP-1 can be tested to quantitatively confirm viruria. Viruria has been observed in 28-40% of renal transplant recipients. The quantity of DNA in the urine is usually 100-fold that of the plasma [72]. Detectable virus in the blood is more predictive of PVAN than viruria alone. Qualitative virus detection in urine by PCR offers little clinical significance while in one study of viral quantitation, BK VP-1 mRNA detection of more than 650,000 copies/ng of total RNA correlated with clinical disease [73]. Some centers prefer urine cytology as the primary screening technique. Decoy cells in urine are a sensitive way to detect overt PVAN. PCR is four times more sensitive than urine cytology for monitoring asymptomatic viruria and provides a more objective estimate of true viral load. It can distinguish BK viruria from JC viruria.

Plasma. In contrast to urine excretion, BK virus plasma viremia is a useful maker of early infection and reflects active viral replication. BK viremia has a negative predictive value of 100% and positive predictive value of 50-82% for development of PVAN [15]. BK viremia develops four-seven weeks after BK viruria and 12 weeks before cytopathic changes are seen in PVAN [15]. BK viremia can be seen in <15% of renal transplant recipients [74], a finding superior to decoy cell screening. Nevertheless, decoy cell shedding can be detected prior to developing viremia. Hirsch et al. found circulating plasma BK DNA of more than 7,700 copies/mL correlated with acute PVAN [15]. In one report, clinical disease was associated with the detection of viral DNA greater than 10,000 copies/ml and sustained for more than one month [69]. Quantitative PCR correlates directly with active PVAN and is useful to monitor disease activity. During the resolution period, viremia resolves prior to viruria.

The approach to diagnosis of PVAN

Screening guidelines to diagnose PVAN after kidney transplantation include urine cytology, urine BK DNA viral load, or urine VP-1 mRNA load screen.

1. At least, three times monthly for the first two years following transplantation.
2. If allograft dysfunction is detected.
3. With any graft biopsy.

Diagnosing PVAN can be challenging as all pathological findings except viral inclusion bodies can also be found in organ rejection. Moreover, in early stages of PVAN the histological diagnosis is difficult because of the focal nature of BK induced PVAN with the resultant possibility of a false negative
result [75]. In one study, there was a 36% discordance with proven PVAN when more than one core biopsy was obtained [69]. At least, two biopsy cores are recommended and should contain medullary parenchyma. A negative biopsy does not rule out PVAN.

Because of the high likelihood of a “false negative” biopsy result in early stage disease, attempts to detect PVAN as early as possible depend on finding elevated PVAN markers such as serial quantitative BK DNA in urine and serum, and the persistent finding of decoy cells in the urine. Prompt reduction of immunosuppression during an early stage of PVAN can lead to resolution of infection, hence avoiding potential irreversible renal scarring.

**Diagnostic classifications** [10]

1. Possible PVAN is defined as any positive screening test
2. Presumptive PVAN is defined as greater than three weeks of either a BK viral load of more than 10^4 copies/mL in plasma, or more than 10^7 copies/mL of urine, or BK virus VP-1 mRNA of more than 650,000 copies/ng of total urine RNA.
3. Definitive PVAN is confirmed by histology.

All patients with a positive urine cytology or viruria, should have periodic serum monitoring of quantitative PCR for BK. A high serum viral load is a strong indication of an invasive BK virus infection. Prolonged viremia without biopsy proven PVAN is known as the “window” period [76]. In this setting, quantitative urine and serum BK virus DNA should be followed every two to four weeks. Patients who meet presumptive criteria for PVAN should undergo renal biopsy regardless of the serum creatinine level.

**Treatment**

The majority of BK infections is asymptomatic, and requires no treatment. HC with a grade of 2 or greater is associated with a higher morbidity and prolonged hospitalization, requiring closer attention and medical intervention. In untreated, immunosuppressed patients, spontaneous resolution of viral replication occurs within 4 to 12 weeks [77].

Clinical experiences with several drugs that are active in vitro have been described in individual case reports or small case series. Consequently, dosage and treatment regimens are not well established. To date, no anti-BK therapies are approved nor licensed.

Several agents have been shown to inhibit polyoma viral replication in vitro. However, there are no published well-designed case control studies regarding the treatment of BK induced HC. In 2000, the first case report of antiviral therapy evaluated cidofovir, an acrylic nucleoside phosphonate analogue, against BK-induced HC in HSCT patients with concomitant CMV reactivation [78]. Later it was successfully used to treat PVAN. Cidofovir was later used for both BK reactivations in solid organ transplants and in HSCT recipients. Subsequently, the value of vidarabine in HC and PVAN was suggested for therapy [79].

**PVAN**

**Manipulation of immunosuppressive therapy**

Reducing, altering, or discontinuing immunosuppressive therapy (therapies) may be an option to control BK infection [28, 80]. These approaches may enable the host immune system to regain control over the infection. Moreover, most immunosuppressive therapies are also nephrotoxic. The reduction of calcineurin-inhibitor dosage is recommended when applicable. Several studies have published successful outcomes by altering immunosuppressive therapies [15].

Stopping MMF or azathioprine in cases of “presumptive PVAN” allowed for clearing of BK viruria and viremia without adversely affecting graft function [74].

Degree of immunosuppression, rather than the immunosuppressive agent, is the critical factor. Two studies demonstrated successful resolution of BK viremia with early reduction of immunosuppressive therapy [74]. Close monitoring for rejection after reduction of immunosuppression is warranted.

Reduction of immunosuppressive drugs in the setting of simultaneous BK infection and acute rejection is controversial. A proposed two-step approach beginning with anti-rejection treatment with pulse methylprednisone, followed by modification, or lowering of maintenance immunosuppression two weeks later has shown favorable outcomes [81]. Immunological clearance of PVAN requires 6 to 24 weeks, whereas stabilization of acute rejection requires 5 to 10 days. Thus, treatment of acute rejection should be targeted first.

**Cidofovir**

Cidofovir has shown significant inhibition of replication of murine and simian polyoma viruses in
vitro, and in clinical studies. Dosage and guidelines for use of cidofovir have not been established. Anecdotal results are conflicting [82]. The basis of anti-BKV activity for cidofovir is not readily understood. DNA polymerase, the primary target of cidofovir for CMV infection is also encoded in the BKV genome. One possible mechanism involves inhibition of a functional domain of BKV’s large T antigen, which is responsible for viral replication [83]. Cidofovir was initially used as a treatment for PML in HIV patients, albeit not very successfully.

Cidofovir accumulates in the renal tubules contributing to nephrotoxicity. Because BK does not encode DNA polymerase, cidofovir must damage replicating host cells that are infected with BK. For this reason a dosage lower than what is used for CMV infection is advocated. Cidofovir is effective when administered at a dose of 0.25-0.33 mg/kg without probenecid. This is one-tenth of the dose used for CMV infection [84].

Leflunomide

Leflunomide is approved as an immunosuppressive therapy for rheumatoid arthritis. One particular metabolite of leflunomide inhibits protein kinase activity and pyrimidine synthesis. This drug also has anti-BK virus activity in vitro. Accompanied by reduction of immunosuppression, leflunomide was successful at a dosage of 100 mg/day for three-five days followed by 20-60 mg/day, to target a serum level of 40-100 g/mL [85]. Given its lack of nephrotoxicity, it may become an alternative to cidofovir. Liver toxicity and BM suppression are serious adverse reactions seen with leflunomide. It appears especially promising because in addition to its anti-BKV activity, its immunosuppressive activity leads to a lower incidence of graft loss than other therapies for graft rejection (15% vs. 35-65%) [86].

Intravenous immunoglobulin (IVIG)

IVIG binds and removes BKV in vitro. However, due to its broad spectrum of activity in vivo, including neutralizing activity and immunomodulatory effects, its role with respect to BK virus therapy is controversial. Potential benefits must be weighed against its serious adverse reactions. The most common and significant reactions include headache, allergic reactions, and aseptic meningitis. IVIG was found to be of clinical value for treating BK infection when combined with reduction of immunosuppression. However, the contribution of these two modalities to treatment response cannot be separated.

Vidarabine

Vidarabine has been used, successfully to treat HC in HSCT patients at a dosage of 10mg/kg administered intravenously once daily for five days [87]. However, the toxicity and lack of availability of vidarabine precludes its use.

Other therapies

Other treatment regimens for BK infection include amantadine and ribavirin but there is insufficient data to establish their value [82].

Re-transplantation

Kidney re-transplantation has been effective for management of PV AN with or without the removal of the infected graft. Recovery of immune function in the absence of immunosuppressive therapy may also explain the clearance of active BK infection in many instances. In multiorgan transplantation, withholding immunosuppressive therapy is not an option. In such cases, nephrectomy may be required to terminate the nidus of BK infection. However, the frequency of recurrence of PVAN within the new graft is 15%.

Hemorrhagic cystitis

Supportive treatment

Low-grade hematuria in HSCT recipients usually resolves spontaneously within two weeks with supportive care alone [88]. Considerations include: 1) hydration to decrease the length of contact between acrolein and bladder epithelium, 2) monitoring drug levels, 3) frequent voiding, and 4) mesna administration [49]. Bedi et al. were unable to find a difference between these preventive measures when managing HC [47]. Other treatment options include analgesics and platelet transfusions. Oral conjugated estrogens (1.5 to 5 mg/day) can be considered for female patients who develop HC.

There is no standard protocol for decreasing immunosuppression in HSCT recipients with HC.

Anti-BK therapy

Cidofovir. Similar to treatment of PVAN, cidofovir 5mg/kg once weekly for two weeks was also used to treat HC. This regimen of cidofovir was effective in a patient with simultaneous CMV and BK HC [78]. Clinical response occurred one week after the first
dose with a reduction in BK viruria followed by improvement in symptoms. Several experimental treatments have been published with favorable and unfavorable outcomes. Currently there is no standard treatment for BK-associated HC [63, 89, 90].

Inspired by a successful trial of instillation of cidofovir into the bladder (in order to minimize systemic side effects) in a patient with adenovirus HC [91], a similar trial was attempted in the treatment of BK HC. This regimen resulted in improvement of hematuria within three days and reduction of viruria [92].

**Fluoroquinolones.** Since the late 1980’s, antiviral effects of fluoroquinolones were established in vitro [93]. The mechanism of action was theorized to be through inhibition of helicase activity of the large T antigen, which has a function similar to DNA gyrase in bacteria, resulting in inhibition of viral DNA repair and replication. The largest case report of fluoroquinolone use in HSCT recipients was performed using ciprofloxacin as prophylaxis, showing lower levels of BK DNA in urine and less reactivation of BK than when receiving cephalosporin prophylaxis. However, when compared with cidofovir, ciprofloxacin was relatively ineffective in inhibiting BK replication [68].

**Leflunomide.** Leflunomide showed a reduction of BK DNA in the urine in 25 out of 37 patients with HC in one report [68].

**Vidarabine.** Vidarabine 10mg/kg intravenously daily for five days showed some success for the treatment of HC [78].

**Urologic intervention**

Urological interventions, such as bladder catheterization with or without continuous irrigation, help in cases of uncontrollable bleeding and prevent recurrent clot retention. Intravesicular instillation of anti-inflammatory or hemostatic agents, such as alum, formalin, or prostaglandin E1, has resulted in mixed outcomes. Cystoscopy with coagulation and laser vaporization produces temporary hemostasis. In life-threatening hemorrhage, cystectomy may be considered.

**Radiological intervention**

Percutaneous arterial embolization (PAE) of vesicular arteries has been used for intractable bleeding due to radiotherapy, tumor, and trauma. Three cases of HC following HSCT were successfully treated with PAE [94]. This option should be reserved for severe HC refractory to conventional therapy.

**Summary**

Our ability to treat BK virus infection lags far behind our knowledge of diagnosis and pathophysiology. Given the low incidence of this infection and its relatively benign nature, therapeutic trials are rare. The existing literature is insufficient to provide treatment guidelines for BK virus infection. With the increase of solid organ and HSCT transplantations, more patients will be at risk. For now, the emphasis is on early detection and modification of immunosuppression when possible. Until less nephrotoxic agents with anti-BK activity are developed, cidofovir will remain the drug of choice.

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**References**


32. Nickeleit V, Singh HK, Mihatsch MJ. Polymavirus nephropathy: morphology, pathophysiology, and


78. Held TK, Biel SS, Nitsche A, Kurth A, Chen S,


