Histologic *versus* Molecular Diagnosis of BK Polyomavirus–Associated Nephropathy: A Shifting Paradigm?

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Although discovered in 1970 the BK virus infections had no significant clinical impact until the emergence of BK virus–associated allograft nephropathy (BKPVAN). Escalating clinical challenges required better diagnostic tools and delineation of uniform criteria for diagnosis. In recent years, the widespread use of real-time PCR for measuring viral loads has confirmed that BK viruria and viremia are consistently identified before the development of overt nephritis. The identification of this viruria-viremia-nephritis sequence has provided tools for screening renal transplant patients and the possibility of earlier intervention with improved outcomes. Analysis of current clinical trends indicates that despite the fact that a positive renal biopsy is the “gold standard” for the diagnosis of BKPVAN, clinical interventions often are based on the surrogate markers of the disease rather than on tissue diagnosis. This is conceptually supported by the fact that early BKPVAN is focal and liable to tissue sampling errors. Strong arguments remain, however, in favor of retaining the requirement for tissue evaluation in patients who are suspected of having BKPVAN. BKPVAN selectively affects the graft and is likely to occur in a background of immune and/or nonimmune renal injury. A renal biopsy is necessary to exclude other pathologic processes (e.g., acute rejection) that could coexist with BKPVAN or be the main cause of allograft dysfunction. Evaluation of a renal biopsy for the purpose of staging is important for prognosis and is also of paramount importance for the rational assessment of therapeutic success.

Renal transplantation became feasible in the early 1960s when a combination of azathioprine and steroids was proved to be successful in suppressing the immune response (1). Shortly thereafter, renal transplant recipients were shown to have a high incidence of viral infections, mainly as a result of cytomegalovirus (CMV) and other herpes viruses. Virologic and serologic studies demonstrated that these could be either primary or reactivated latent infections with a wide spectrum of clinical severity (2,3).

In 1970, during a systematic study of viral infections in renal transplant recipients, the BK polyomavirus was identified (4). This pathogen was found to have very high homology with the JC virus, the other human polyomavirus virus that was discovered around the same time as the etiologic agent of progressive multifocal leukoencephalopathy (5).

Spectrum of Clinicopathologic Manifestations

The BK and JC viruses are ubiquitous and cause trivial infections in childhood. After the primary infection, both the BK and JC viruses remain latent in the renal tubular epithelium, where viral replication is controlled by mechanisms of immune surveillance. Viral replication with shedding of viral particles in the urine (viremia) occurs only when the immune functions are impaired (6). Physiologic fluctuations of the immune functions (e.g., older age, pregnancy) may be associated with viral replication manifesting as viruria, but these events have no clinical significance (6). In contrast, in some patients with severe immune dysfunction, the viral replication proceeds unrestricted, leading to structural organ damage and dysfunction (e.g., cystitis, ureteritis, nephritis) (7). Tissue damage results from a combination of direct viral cytolysis and secondary inflammatory responses. The latter may be immune specific or nonspecific in nature (7,8). The intricate interactions between the virus and the immune system lead to various clinicopathologic forms of BK disease, as has been schematized previously by Hirsch (9). Whereas viral replication may proceed without apparent engagement of the immune response (e.g., BK virus–associated allograft nephropathy [BKPVAN] histologic pattern A, JC in progressive multifocal leukoencephalopathy), the BK infection often triggers the development of a vigorous immune response that may lead to viral clearance (i.e., lymphoid infiltrates in BKPVAN histologic pattern B) (8,9). Excessive or aberrant immune responses to the BK virus may result in diseases such as hemorrhagic cystitis in bone marrow transplantation and autoimmune disorders, respectively (9).

With respect to renal transplantation, the factors that influence the progression from limited viral replication to BKPVAN are not completely known. It is clear, however, that in the backdrop of immunosuppression, a variety of factors, including ongoing graft injury as a result of allograft rejection, ischemia, and/or drug toxicity, increase vulnerability of the graft to the
BK infection. Other factors, such as host or host–graft relationship variations (e.g., HLA type, HLA match/mismatch) and viral genomic changes (e.g., mutations, rearrangements), also have been implicated (6,7).

**BK Virus and Renal Transplantation: Historical Perspective**

There is ample epidemiologic and virological evidence that the BK human polyomavirus has co-evolved and adapted to its human host over a long period of time (9). Despite its widespread distribution, the BK virus was not known to cause disease before the systematic use of immunosuppressive treatment for renal transplantation (10). Cells with polyomavirus-like cytopathic changes had been observed earlier in the urine of patients who were treated with cyclophosphamide, but their nature was not known then (11).

The first patient who was found to have a BK virus infection was receiving maintenance azathioprine and prednisone immunosuppression for a renal transplant that he had received 3.5 mo earlier (4). At the time of diagnosis, he presented with anuria and pain over the graft. An intravenous pyelogram revealed a distal ureteric obstruction that was corrected surgically. Histologic evaluation of the resected ureteral segment demonstrated intranuclear viral inclusions in the urothelium, with granulation tissue and fibrosis of the wall. Large numbers of cells with intranuclear inclusions were present in the urine. These cells are known as “decoy cells” because of their potential misidentification as malignant cells (8). After surgical correction of the stenosis, the patient recovered satisfactorily and had excellent renal function 7 mo after the diagnosis of BK ureteritis (4).

The association between renal transplantation and shedding of polyomavirus in the urine was established firmly by subsequent studies (10,12–14). The reported incidence of viruria in patients who received azathioprine and prednisone ranged from 14 to 68%, depending on the method used to demonstrate the infection (10,12–16).

From the morphologic point of view, the cytopathic effects of the BK virus were soon well characterized in urine samples and in histologic material from several patients with ureteral stenosis (4,10,12–16). Because of the scarcity of renal biopsy samples in the studies published before 1995, it is unclear whether the high incidence of symptomatic ureteritis that was reported during this period represents a real clinical trend or tissue sampling bias by the availability of histologic material in pa-

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In earlier studies, the overall clinical impact of the BK infections is difficult to determine because clinical details and outcomes are to some degree overshadowed by the morphologic, virologic, and serologic descriptions. In the report of Mackenzie et al. (16), all seven patients who were shedding decoy cells in urine had previously required treatment for acute rejection. Graft dysfunction was present in the three patients with histologically confirmed BK infection. Of these three patients, one underwent a nephrectomy a few days after the diagnosis, one died a few weeks later, and the third one (see above) was alive and well 5 mo after the diagnosis.

Two additional comprehensive prospective studies that were published in the 1980s further characterized the patterns of JC and BK infections in renal transplant recipients (13,14). Serologic evidence of active polyomavirus infection was found in 41 to 65% of patients. Both primary and reactivated BK and JC infections were identified, with a predominance of reactivated infections in the patients with BK virus (13). These studies indicated that clinical and histopathologic evidence of BK disease was rare in contrast to the common incidence of infection and viruria (12–15). A negative impact of the polyomavirus infections was suggested in the study of Hogan et al. (14), in which polyomavirus replication was associated with increased creatinine levels and an overall increase in transplant-associated complications.

The diagnosis of polyomavirus infection before 1995 was based on the demonstration of rising antibody titers, cyologic evaluation of urine sediment, viral isolation from urine, electron microscopic and immunoelectron microscopic studies of urine, immunofluorescence staining of urine sediment with antibodies against BK and JC viruses, and routine evaluation of tissue sections (4,10,12–17).

**BK Virus Infection under Current Immunosuppression Regimens**

Maintenance immunosuppression regimens for renal transplantation were significantly strengthened with the introduction of triple drug combinations that typically consisted of a calcineurin inhibitor (cyclosporine or tacrolimus), mycophenolate mofetil, sirolimus, and prednisone. The global increase in immunosuppression resulted in a marked reduction of acute rejection episodes but also caused a significant increase in clinically evident BK infections, in particular BKPVAN (18).

The first bona fide case of BKPVAN characterized by the typical viral cytopathic effects in renal tubular epithelium and graft dysfunction was reported in 1995 (19). Later studies that were based on systematic evaluation of serial biopsies and nephrectomy specimens outlined the morphologic characteristics of disease progression. Parenchymal involvement in the earlier stages of the BKPVAN disease is randomly focal. Early disease is characterized by viral cytopathic changes and lack of significant associated inflammation and atrophy. Progression is characterized by centrifugal enlargement of the infected foci, eventually leading to confluent areas of scarring (tubulointerstitial atrophy and fibrosis) that are typically accompanied by chronic inflammation. Three morphologic patterns of BKPVAN are encountered in renal allograft biopsies that encompass the spectrum of early (pattern a), intermediate (pattern b), and late (pattern c) lesions (7). Patterns a, b, and c are characterized by viral cytopathic changes, inflammatory response, and extensive fibrosis, respectively. The histologic pattern that was found in the first diagnostic biopsy with BKPVAN has been reported to
correlate with ultimate graft outcome (20). Biopsies with a more advanced pattern (more tubulointerstitial fibrosis and inflammation) are typically seen in patients with abnormal renal function (20,21).

Inflammatory infiltrates are inherent to BKPVAN, and active tubulointerstitial inflammation (tubulitis) at the time of diagnosis is associated with an increased incidence of graft loss (20). The identification of tubulitis poses a difficult diagnostic question because BKPVAN can develop in patients with previous or ongoing acute rejection and currently there are no tools to differentiate conclusively pure viral nephritis from acute rejection (22). The identification of significant tubulitis in a biopsy that shows BKPVAN is considered by some as an indication for antirejection treatment (i.e., pulse steroids) before maintenance immunosuppression is decreased (23).

Parenchymal scarring (tubular loss, atrophy, and fibrosis) remains as sequela of BKPVAN even when the infection is cleared after reduction of immunosuppression (20). Depending on the extent of parenchymal scarring, shortened graft survival may occur even with prompt clearance of infection after immunosuppression is decreased. A successful outcome in BKPVAN is characterized by stabilization or improvement of the creatinine level, disappearance of viremia, disappearance or very marked reduction of viruria, and absence of viral cytopathic changes in follow-up biopsies (20). Comparison between the degree of scarring and inflammation in baseline and subsequent biopsies would be necessary to assess the potential usefulness and/or toxicity of any new therapeutic agent to treat BKPVAN.

The difficulties in the morphologic diagnosis of BKPVAN, in particular with respect to the differentiation from acute rejection, became apparent as an alarming number of cases were reported worldwide (24). For avoidance of the potential for errors particularly in the form of overdiagnosis, there was general agreement that the diagnosis of BKPVAN required the histologic demonstration of the polyomavirus cytopathic changes and confirmation with additional studies such as immunohistochemistry (25).

More recently, it has become evident that contrary to the initial impression, the diagnosis of allograft BK infections is complicated by the wide gamut of clinicopathologic changes. These correspond to the spectrum of renourinary involvement that ranges from focal, episodic, and inconsequential to extensive, progressive, and potentially leading to accelerated graft loss (9,20,26).

Of particular importance for the understanding of the evolution of BKPVAN was the prospective study of Hirsch et al. (23) demonstrating that histologically proven BKPVAN is consistently preceded by a period of asymptomatic viruria and the development of viremia. Furthermore, their study showed that BK viremia of \( \geq 10^4 \) is characteristic of patients with biopsies that show BKPVAN (23). Additional clinical and histologic studies in larger numbers of patients have confirmed that high-level viral replication that leads to nephritis is constantly associated with viruria and viremia (20,27,28). The recognition of the succession of events that lead to BKPVAN (viruria-viremia-nephritis) has provided the rational basis for screening protocols to identify patients who are at risk (18,29–38).

### Screening for Prevention of BKPVAN

Current guidelines for the screening and diagnosis of BKPVAN have been outlined by a multidisciplinary international panel (18). The main points of these guidelines are summarized in Figure 1. Screening for viruria should be done every three months in the first 2 yr posttransplantation and yearly thereafter. Viruria can be studied either through evaluation of exfoliative urine cytology (decoy cells) or with molecular studies for quantification of the viral load (18,24,30). Cytology examination is less sensitive than the molecular-based methods and cannot differentiate between the BK and JC viruses. In the clinical setting, however, urine cytology is an excellent screening method to demonstrate viruria. Persistence of BK virus shedding (decoy cells or DNA loads >10^7/ml) rather than isolated/episodic detection has been shown to identify patients who are at risk for BKPVAN (18,27). Although viral replication in the renourinary tract (expressed as viruria) is observed in 30 to 40% of patients in the first 6 mo posttransplantation, significant viremia develops in significantly fewer patients (12 to 15%), and further progression to BKPVAN is even more rare (≤10%) (30,32–35).

A positive screening test (presence of decoy cells or BK urine load of >10^7/ml) should prompt evaluation of viremia, which if present for 3 wk or more likely indicates progression to BKPVAN (18). In patients with significant viruria, quantifica-

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**Figure 1.** Recommended algorithm for screening, diagnosis, and follow-up of BK allograft nephropathy.
tion of BKV viremia has emerged as the most specific surrogate marker indicating renal parenchymal involvement (nephritis). Serial evaluation of viremia is also the best tool to demonstrate resolution of the disease after immunosuppression has been decreased (18,20,23,24,28,34–38). However, determination of viremia alone is not cost effective for screening because of the low sensitivity of this test (70%) (18,30). Serial determinations of viruria and viremia also are required in the evaluation of patients who are considered for retransplantation after losing graft function to BKPVAN (39).

Early Diagnosis of BKPVAN

Because of the potential for causing irreversible renal scarring, early diagnosis of BKPVAN is now considered one of the main factors that can lead to resolution of the infection and a successful outcome after decrease in immunosuppression (40). Early stages of the disease are difficult to diagnose histologically because of the focal nature of BKPVAN and the resulting possibility of having only uninvolved areas represented in the biopsy core (8,18,20). The negative impact of a falsely negative biopsy is avoided when the renal biopsy is interpreted in the context of viruria’s and viremia’s serving as surrogate markers of BKPVAN. This approach allows for an accurate and early diagnosis before there is significant parenchymal damage. The term “presumptive BKPVAN” has been coined to identify patients with (1) significant viruria—indicating viral replication in the urinary tract—and (2) viremia of ≥10^4 viral copies/ml for >3 wk (18).

In practice, several clinical approaches are used for the prevention and early diagnosis of BKPVAN using the molecular and histologic methods that currently are available. The main screening protocols are summarized below. To a large extent, these protocols reflect the needs and practices of the various centers where they have been developed (23,26,35,41).

Ramos et al. (26) successfully used a screening protocol that is based on the prospective evaluation of urine cytology. According to this protocol, persistence of decoy cells in urine for 3 mo or more triggers the performance of a renal biopsy independent of the renal function. The renal biopsy findings in combination with concurrent and subsequent measurements of viremia determine the therapeutic intervention. Immunosuppression reduction is progressive and tailored to the needs of the individual patient (e.g., previous history of acute rejection, simultaneous pancreas transplant). In a preliminary study, early diagnosis with this method led to viral clearance in a large proportion of patients and markedly reduced graft loss (26,29).

Hirsch et al. (23) screened patients with urine cytology for decoy cells every 3 mo. A positive urine cytology triggers additional studies, including determinations of viremia and renal biopsy.

Brennan et al. (35) proposed preemptive reduction of immunosuppression in patients with significant and sustained viruria and viremia. Immunosuppression reduction consists of discontinuation of the antimetabolite drug (e.g., mycophenolate mofetil) with further reduction in calcineurin inhibitor if viremia persists. In their study of 200 patients, resolution of viremia occurred in 95% of the 23 patients treated. Renal biopsies were performed only in selected cases; histologically proven BKPVAN was not identified, suggesting that its occurrence was prevented.

Buehrig et al. (41) used routine surveillance biopsies to identify patients with silent BKPVAN and demonstrated improved outcomes compared with patients who had allograft dysfunction at the time of diagnosis.

Conclusion

For renal transplantation, all of the major allograft processes (e.g., acute rejection, chronic allograft nephropathy, acute tubular necrosis) are addressed with the use of renal biopsies as the diagnostic “gold standard.” Similarly, for BKPVAN, evaluation of a renal biopsy provides the only means for assessing the disease pattern, including the extent of renal scarring, the type and the extent of the immune response, and the existence of other, concurrent pathologies (8,18,20).

Patchy involvement of the renal parenchyma by any renal disease has been recognized as a source of diagnostic errors (e.g., underdiagnosis) in needle biopsies (20). Fortunately, in the case of early, typically focal BKPVAN, the concurrent determination of viral loads that are released in body fluids (viremia and viruria) circumvents any possibility of misdiagnosis as a result of sampling errors.

Prospective evaluation of renal transplant recipients can identify patients who are at risk for developing BKPVAN. Early diagnosis that is based on a combination of tissue and molecular studies has resulted in marked improvements of the outcome despite the current lack of specific treatment.

References


