The influence of immunosuppression on the development of BK virus nephropathy—does it matter?

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Abstract
In the last decade the incidence of BK virus infection has increased in renal transplant recipients and become an important factor negatively influencing graft outcome. BK virus infection cannot be attributed to a single immunosuppressive agent or regimen. The risk of BKV infection is related to the overall load of immunosuppression, which is determined not only by immunosuppressive drugs but also by the humoral and cellular immunity of the recipient. Reduction in immunosuppression at this time appears to be the best available approach to the treatment of established BKVN. Assays are lacking that are able to measure the degree of immunosuppression in a given patient at a given time after transplantation. The balance between a sufficient yet nontoxic immunosuppressive regimen remains a major problem in preventing complications such as BK virus nephropathy. This article will focus on the influence of immunosuppressive medication on the development of BKVN. The role of other aspects such as viral virulence, humoral and cellular immunity or renal specificity will be shortly discussed.

Keywords: BK virus nephropathy; BK virus nephritis; polyomavirus; renal transplantation; immunosuppression

Introduction
The BK virus belongs to the family of polyomaviridae and represents one of two types of human polyoma viruses. Polyomaviridae have a 5 kb genome of small circular double-stranded DNA. BK virus as a clinical pathogen in patients after renal transplantation was first detected in 1971 [1] but it took another 25 years until an increasing incidence of BK virus nephropathy (BKVN) was observed in transplant centers worldwide. In the last decade the incidence of BK virus infection has increased in renal transplant recipients and negatively influences graft outcome [2–4]. About 80% of the general population have detectable antibodies to BKV [5], antibodies typically appear in childhood and remain elevated throughout life. BKVN prevalence has been reported between 1–9% in adult and pediatric patients. In a prospective study from Hirsch et al. the prevalence of BK viruria, viremia, and BKVN after renal transplantation was 30, 13 and 8%, respectively [6]. BKVN following other solid organ transplantations is a rare phenomenon, BKVN of the native kidneys was occasionally described in HIV patients and patients with immunodeficiency syndromes. The typical clinical manifestation of BKVN is renal graft dysfunction. As well BKVN can be detected in routine protocol biopsies in the absence of graft dysfunction. In rare cases ureteric obstruction or cystitis is the leading clinical feature. Progressive renal failure has been reported in approximately 30–60% of cases [3]. Recently improved graft survival has been reported from centers with screening programs allowing early diagnosis of BKV replication and subsequent intervention [7–9].

The diagnosis of BKV infection is based on the detection of viral cytopathic effects represented by urinary decoy cells and the virus itself which could be found in urine, blood and renal tissue. The presence of BKV-specific antibodies reflects immunity to the virus. Histologic evaluation of the kidney in BKVN typically shows nephritis. Urinary decoy cells are a valuable diagnostic screening test, but the positive predictive value of a positive decoy cell analysis to predict BKVN is low with around 20–30%, however the negative predictive value is about 99% [10]. Therefore urinary decoy cells suggest the presence of BKV in urothelium, but they are non-specific and do not predict BKVN. Viruria can be documented in 28–35% of renal transplant recipients [6,11]. The presence of urinary BKV DNA as a diagnostic tool has a good negative predictive value but a poor positive predictive value (40%). A recent study suggests that amplifying viral VP1 mRNA in the urine might offer a better diagnostic approach, as it may represent active BKV.
Pathogenesis of BK-virus nephropathy

The are several mechanisms involved in the pathogenesis of BK virus nephropathy. The exact pathogenesis leading from BKV infection to BKVN remains unclear. Concerning the source of BKV infection two scenarios are discussed. In patients who have never been exposed to or acquired BK virus, especially in pediatric recipients, transmission may occur through the donor kidney [13]. The study of Bohl et al identified donor seropositivity as an important factor in the occurrence of BKV infection [14]. Most adult patients (about 80%) have been exposed to the virus prior to transplantation resulting in the production of antibodies. Therefore the second hypothesis is that after transplantation latent BKV infection in the renal epithelium is reactivated. Location of latent virus not only in the renal epithelium but in ureteral and bladder mucosa may be possible.

The exact factors leading to reactivation of BKV remain unclear. A major role might play the defective immune competence in the immunosuppressed recipient. From other viral infections we have learned that the host humoral and cellular immunity play an important pathogenic role. Lack of development of BKV-specific IgG may be a key feature in the manifestation of BKVN [15]. Seronegative patients are at increased risk for BKV replication and BKVN whereas patients who have prior immunity to BKV may not develop infection at all. Patients with a high viral load and sufficient humoral immunity may not require any intervention, patients with a low viral load and no detectable anti-BKV immunity may be in risk of developing BKV disease. Independent of humoral immunity patients with elevated BKV-specific antibodies are still able to develop BKVN, suggesting a role of defective cellular immunity [6,16]. In patients with BKVN BKV-specific T-cells could not be detected in peripheral blood but reappeared after clearance of BK viremia. As we know effector functions of T lymphocytes are critical to viral immunity. Lack of cellular immune responses to elevated viral loads might determine the occurrence of BKVN.

The mode of activation of BKV and mechanisms through which BKV infects renal tubular epithelial cells in transplant recipients are unknown. BKV shows tropism to renal tubular epithelial cells and replication occurs in renal epithelium. The receptor site for BKV still has to be identified. It remains unclear why BKV infection occurs predominantly in renal transplants. Host cell regeneration after tubular cell epithelium injury might be an important cofactor [17]. Ischemic injury at the time of transplantation may play a role in creating an environment for viral replication. Some investigators have postulated a double-hit hypothesis, in which a combination of preservation injury and immunologic damage predisposes renal grafts to BKVN. On the other hand development of BKVN a year after transplantation questions the role of ischemic injury as a single important factor for BKVN.

Alltogether the pathogenesis of BKVN is complex, as the virus may be introduced into the recipient through the donor, or reactivation in the recipient, or both. Tropism of this virus for renal tubular cells is the basic principle for infection, being modified by renal epithelium injury and host humoral and cellular immunity, with immunosuppressive therapy finally leading to BKVN.

Influence of immunosuppression on BK-virus nephropathy

Type of immunosuppressive regimen

BKVN has been diagnosed in patients receiving a maintenance therapy consisting of different drug classes like calcineurin inhibitors, antimetabolites, mTOR inhibitors and corticosteroids. Due to the fact that prior to 1995 BKVN was rarely identified as a clinical problem in renal transplantation the role of changing immunosuppressive regimens as a causative factor in the occurrence of BKVN has emerged. In the mid-nineties Mycophenolate and Tacrolimus were introduced as new immunosuppressive agents in transplantation medicine. In maintainance immunosuppression the use of Tacrolimus (FK506) compared to Cyclosporine (CyA), or Mycophenolate compared to Azathioprine, has been implicated as a major determinant of BK viruria, viremia and BKV replication [12]. Circulating BKV DNA in plasma has been found in approximately 10–15% of renal transplant recipients and developed 4–7 weeks after BKV viruria. Not all patients with viruria present with clinical BKVN. Detection of viremia by PCR is a reliable test for BKVN, as it is seen in nearly 100% of cases with BKVN. However, the positive predictive value for BKVN is only 60%. The exact levels of circulating plasma BKV DNA correlating with BKVN remain controversial. A number of copies >7000 was correlated with acute BKVN [6]. Plasma viral loads of >1 x 10^4 copies/ml have a positive predictive value of greater than 80%. However, in single cases BKVN has been demonstrated even with copies below this threshold and 50% of all viremic episodes are only transient. Histological diagnosis represents the gold diagnostic standard. The final diagnosis of BKVN is made by demonstration of viral cytopathic effects in renal biopsy. Typical findings are focal interstitial mononuclear cell infiltrates, plasma cells, tubular necrosis and intranuclear inclusion bodies [8]. Histopathology can be misleading as renal involvement can be focal in early BKVN and could present with advanced fibrosis and minimal inflammatory changes in later stages. It should be mentioned that interstitial inflammation with BKVN is difficult to differentiate from acute rejection. Immunohistochemistry with SV40 staining is now routinely used to document the presence of BKV in renal tissue.

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nephropathy [3,18,19,20]. An increased risk of Tacrolimus-MMF-corticosteroid combinations for BKV replication and BKVN was demonstrated in a prospective study [7] and in a retrospective histopathology study [19], respectively. In some centers cases of patients with BKVN have been described after switching from cyclosporine to tacrolimus in a dual regimen with steroids as a rescue attempt after rejection [2,4,21].

In 2001 Barri et al. reviewed their experience in 8 patients with BKV infection (three year period, 161 renal transplants) [20]. All patients received MMF as part of their immunosuppressive protocol and 7 of 8 patients were on tacrolimus. In their 2003 published study Mengel et al. [19] retrospectively investigated their renal transplant biopsies performed in a three year period (n = 1276). They correlated the results to the different immunosuppressive protocols used and patients with BKVN were compared to a matched control group. BKVN was identified as a rare (7/638 patients; 1%) but serious complication. Four out of seven patients with BKVN were under triple immunosuppression with tacrolimus, MMF and steroids. With this immunosuppressive regimen an eight times higher incidence and a thirteen times higher risk of developing BKVN compared with the control group was observed. Looking at the combination of two potent immunosuppressive drugs, not all patients receiving MMF and/or FK506 developed BKVN. Patients receiving MMF as part of a double immunosuppressive maintenance regimen with MMF and steroids (n = 20) did not acquire BKVN and only 2 out of 197 patients with triple immunosuppression including MMF developed BKVN. No cases of BKVN occurred in patients receiving FK506 without MMF (n = 77) but in 4/50 (8%) patients with triple immunosuppression with FK506, MMF and steroids. No BKVN was observed under a routine triple immunosuppression with CyA, MMF and steroids.

Brennan et al. prospectively evaluated the differences between viremia, viruria an BKVN with three different immunosuppressive regimens [7]. Patients were randomly assigned to receive FK506 (n = 134) or CyA (n = 66), as second agent patients routinely received azathioprin (AZA). MMF (n = 88) was substituted for AZA (n = 112) under certain circumstances (e.g. second transplant, PRA >20%) and all patients received prednisone tapered by month 3 to 5–7.5 mg daily. By year one 35% of patients developed viruria and 11.5% viremia, neither were affected independently by FK506, CyA, AZA or MMF. Their study revealed no difference in the rate of BK viruria or viremia among those receiving FK506 compared to CyA. As well no differences were found with AZA compared to MMF. The incidence of sustained viremia, however, tended to be higher with FK506 than CyA. Of the four possible combinations of calcineurin inhibitor and antimetabolite the CyA-MMF combination was associated with the lowest incidence of viruria and viremia and FK506-MMF with the highest. Viruria was highest with FK506-MMF (46%) and lowest with CyA-MMF (13%) (P = 0.005). It should be noted that CyA lowers MMF levels whereas FK506 does not, but no data were provided concerning mycophenolate blood levels. This study illustrates that BKV infection is not specific for certain immunosuppressive agents but the FK506-MMF combination is the most permissive regimen for BKV reactivation.

In a 2005 published retrospective single-center analysis Vasudev et al. [22] evaluated prevalence, risk factors, timing, and outcome in renal transplant recipients with BK virus nephritis (41 cases out of 1001 renal and renal/pancreas transplant recipients). No individual immunosuppressive agent was identified as a single risk factor. Because the occurrence of BKVN is thought to be linked to intensity of immunosuppression, for analysis of these cases, the authors applied an empiric immunosuppressive unit scale to quantify the degree of immunosuppression. This approach was then applied retrospectively to the evolution of all BKVN cases with diagnosis based on quantitative blood BKV-PCR and biopsy. One unit of immunosuppression was assigned for each of the following doses of immunosuppressive medications: cyclosporine 100 mg, tacrolimus 2 mg, mycophenolate mofetil 500 mg, prednisone 5 mg, sirolimus 2 mg, and azathioprine 100 mg. Immunosuppression was then quantified in units/day at the time of diagnosis of BKVN, at 1 month, and 3 months’ postdiagnosis of BKVN. The immunosuppressive scale score was 7 units at the time of diagnosis of BKVN and decreased to 3.5 units at 3 months post-BKVN. Reduction in the dose of calcineurin inhibitors but not the overall reduction in dose of immunosuppression correlated with recovery of renal function in these patients. Despite reduction in immunosuppression, graft loss occurred in 46% of patients.

Smith et al. assessed the incidence, risk factors, clinical and virological features of BKVN in a cohort of 173 paediatric kidney transplant recipients [13]. Histologically confirmed BKVN developed in 6 patients. There was no association between any specific induction immunosuppressive agent and BKVN. The initial immunosuppressive regimen was CyA, MMF and steroid (n = 5) and CyA, sirolimus and steroid (n = 1). One patient was switched from CyA to tacrolimus 4 month prior to diagnosis, 4/6 patients had biopsy-proven rejection and steroid pulse therapy prior to diagnosis. However, BKVN has also been seen occasionally in recipients receiving calcineurin inhibitor (CNI)-free immunosuppressive regimens. Patients described in a small series [23] never received CNIs as part of their immunosuppressive regimen and developed BKVN. Two of these patients received an immunosuppressive regimen consisting of an IL-2 receptor antagonist while one received thymoglobulin. All three patients were maintained on prednisone, sirolimus and MMF, none was treated for rejection. In an immunosuppression minimization trial a patient with Thymoglobulin induction, Sirolimus, MMF and steroids also
developed BKVN [24]. If this combination of two antiproliferative agents reflects the overall degree of immunosuppression remains unclear. Mengel et al. reported a case of biopsy-proven BKVN when sirolimus was added to a dual regimen with CyA and steroids [19].

In patients treated with calcineurin inhibitor-based regimens the role of steroids in influencing BK viremia and BKVN requires further investigation. There are studies suggesting that avoidance or early cessation of steroids may be associated with a lower incidence of BKVN. In a retrospective single center analysis of 213 kidney and 14 kidney/pancreas transplants early steroid cessation (≤7 days) or steroid avoidance regimens resulted in a lower incidence of BKVN (0 vs 3.5%) [25]. Analyzing BK virus replication (>6.5 x 10^5 BKV VP1 mRNA/μg total RNA) in 98 consecutive renal allograft recipients, Dadhania et al. found a lower incidence of BKVN replication in the steroid free group, suggesting the contribution of steroid maintenance therapy to BKVN replication [26].

In the presence of BKV nephritis the diagnosis of acute rejection, especially tubulointerstitial rejection (Banff grade I or borderline changes), may be challenging. Inflammatory infiltrates can be seen as a response to allograft antigens as well as to viral antigens. The diagnosis of BKVN and concurrent acute rejection classified according to the Banff criteria leaves the question how to proceed with antirejection treatment. Different studies have shown that the use of i.v. steroids as rejection treatment may increase the risk of development of BKVN as long as immunosuppressive maintenance therapy is continued or even intensified [2,4,6,27]. In their 1999 published article Nickelet et al. [2] reported about the follow-up of 5 cases with persistent BKVN, all of the 5 patients had a complicated posttransplantational course with recurrent rejection episodes. Immunosuppression has been switched from cyclosporin to high-dose tacrolimus rescue therapy and repeated steroid boluses were administered. Hirsch et al. [6] reported about an association between prior rejection episodes and rejection treatment with steroid pulses and an increased risk of viraemia, viremia or BKVN. When factors of borderline significance in the univariate analysis (P<0.1) were included in multivariate regression models, the number of corticosteroid pulses remained significantly associated with BKV viremia (P=0.01; RR 1.28), BKV replication (P=0.01; RR 1.21) and nephropathy (P=0.01; RR 1.38).

In cases of BKVN and concomitant biopsy proven acute rejection a two-step approach of antirejection treatment followed by reduction of maintenance immunosuppression two weeks later is recommended [28]. Several studies have shown that stabilization or improvement of allograft function can be observed when steroid treatment for acute rejection was followed by decreased maintenance therapy [6,20,27].

Another question raises about the association between antilymphocyte preparations and the development of BKVN. Their impact may depend on the type of different available agents and the specific clinical situation they are used with. In induction therapy antilymphocyte preparations could not be identified as an independent risk factor for BKV viremia, viremia or BKVN [4,6,7,18,29].

Looking at the role of antilymphocyte preparations in the treatment of rejection their use can be associated with BK virus replication. In most cases patients received FK506 or MMF as part of a standard or rescue regimen [4,6]. In their analysis of HLA mismatching and risk of BK virus nephropathy Awadalla et al. showed that BK virus nephritis is associated with a greater number of rejection episodes and a higher incidence of steroid-resistant rejection requiring antilymphocyte treatment [30].

Dosing of immunosuppression

Looking at the influence of drug concentration and dosing on the occurrence of BKV reactivation higher doses of tacrolimus (through levels >8 ng/ml) or MMF have been associated with BKV replication and BKVN [19]. Moreover, the reduction of tacrolimus and BKV replication from >9 ng/ml to 6 ng/ml and MMF to a daily dose ≤1g resulted in improvement or stabilization of BKVN in 9 out of 10 cases [31]. In a recent published study from the Mayo Clinic [32] a group of kidney transplant recipients between 2000 und 2002 with high Tacrolimus levels (HiTAC, n=245) was compared to a second group between 2002 and 2004 with lower Tacrolimus levels (LoTAC, n=330). The change in Tacrolimus levels (15% reduction) was made in an attempt to reduce the incidence of polyoma virus nephropathy while other immunosuppressive medications remained unchanged. At one year posttransplant the LoTAC group showed a lower incidence of BKVN (2.5 vs 10.5%) and a higher iohaluminate glomerular filtration rate (59 vs 52 ml/min/m²), on protocol one year biopsies a lower incidence and severity of interstitial fibrosis was demonstrated. The incidence of rejection was similar between both groups. Compared to HiTAC the achieved Tacrolimus through levels were significantly lower in LoTAC patients during the first months (12.7 vs 10.8 ng/ml), from 2 to 4 month (12.1 vs 10.9 ng/ml), 4 to 12 months (8.8 vs 7.5 ng/ml) and from month 12 to 24 (7.7 vs 7.3 ng/ml). The tacrolimus exposure was higher in the HiTAC group (AUC: 4221 vs 3664). No data was provided about MMF exposure in both groups. Altogether a relatively modest reduction in tacrolimus exposure resulted in a 72% lower incidence of BK virus infection. In a recent study from Japan [33] severe BKV infection was found in living donor renal transplant recipients with tacrolimus levels >8 ng/ml and those with an acute rejection episode within the first month after transplantation.

In the prospective study of Brennan et al. [7] evaluating the differences between viremia, viruria and BKVN with three different immunosuppressive regimens tacrolimus through levels were aimed to be
5–10 ng/ml. Identification of BK viremia triggered discontinuation of AZA or MMF, if viremia failed to clear within 4 weeks the tacrolimus dose was tapered to levels of 3–5 ng/ml. Cyclosporin and tacrolimus levels were not different in subjects with or without BK virus early after transplantation. Thereafter the tacrolimus or CyA levels in the BK viremic patients decreased due to the protocol-driven immunosuppression reduction. Reduction of immunosuppression was associated with clearance of viremia in 22 of 23 viremic patients (95%) by 1 year after treatment. The mean time to clearance was 54 days (7–213 days). In 7 patients viremia cleared after cessation of MMF or AZA alone, in 2 patients a decrease in the calcineurin dose alone was made. Six patients required cessation of MMF or AZA and a decrease in the calcineurin dose. Viremia cleared in 7 patients with a single positive plasma BK with standard immunosuppressive tapering and before protocol-driven discontinuation of MMF or AZA was implemented.

Conclusion

BK virus infection can not be attributed to a single immunosuppressive agent or regimen. The risk of BKV infection is related to the overall load of immunosuppression, which is determined not only by immunosuppressive drugs but also by the humoral and cellular immunity of the recipient. Statistically valuable threshold values for drug levels or doses in order to minimize the risk of BKVN have not yet been established. Because save and effective antiviral agents are not available, current therapeutic strategies are mainly based on modifying maintainance immunosuppression. Reduction in immunosuppression at this time appears to be the best available approach to the treatment of established BKVN. Early diagnosis and avoidance of excessive immunosuppression can stabilize the disease in the majority of cases. Stabilization of function may take up to 3 months after changing immunosuppressive therapy. As reducing the level of immunosuppression can lead to acute allograft rejection, prevention of BKVN by monitoring BK viruria and viremia seems to be mandatory. Preemptive reduction of immunosuppression as preventive therapy for BKVN has been successfully performed [7].

Assays are lacking that are able to measure the degree of immunosuppression in a given patient at a given time after transplantation. In the future we should try to identify immune markers for monitoring of the humoral and cellular immune response of the organ recipient. The balance between a sufficient yet nontoxic immunosuppressive regimen remains a major problem in preventing complications such as BK virus nephropathy. Early detection of overimmunosuppression may be of value in avoiding the development of BKVN.

Conflict of interest statement. None declared.

References


