



Diagnostics, treatment, and immune response in BK polyomavirus infection after pediatric kidney transplantation

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Abstract

After pediatric kidney transplantation BK polyomavirus (BKPyV) infections are associated with an increased risk of graft loss by BKPyV-associated nephropathy (BkPyVAN). However, suitable prognostic markers for the individual outcome of BKPyV infections are missing and the management of therapeutic interventions remains a challenge to the success of pediatric kidney transplantation. This review gives an overview on current diagnostic and therapeutic strategies in the field of BKPyV infections after pediatric kidney transplantation. Methods determining the individual immune response to BKPyV are described and their usability is discussed. There is growing evidence that BKPyV-specific T cells (BKPyV-Tvis) may serve as prognostic markers in order to steer immunosuppressive therapy in pediatric kidney recipients with BKPyV viremia in future. Prospective randomized trials in viremic kidney recipients comparing Tvis-steered therapeutic intervention with standard reduction of immunosuppression are needed before implementation of BKPyV-Tvis monitoring in routine care of BKPyV infections.

Keywords BK polyomavirus · BK polyomavirus-associated nephropathy · Virus-specific T cells · Kidney transplantation · Pediatric transplantation · Immunosuppression · mTOR inhibitors · Viral infections · Prognostic marker

Abbreviations

BKPyV	BK polyomavirus
BKPyVAN	BK polyomavirus-associated nephropathy
CMV	Cytomegalovirus
CsA	Cyclosporine A
EBV	Epstein Barr virus
ELISpot	Enzyme-linked immunospot
IVIG	Intravenous immunoglobulin
mTOR	Mammalian target of rapamycin
PCR	Polymerase chain reaction
TAC	Tacrolimus
Tvis	Virus-specific T cells

Introduction

After solid organ transplantation, immunosuppressive treatment disturbs the individual balance between virus replication and cellular immune response resulting in an elevated incidence of severe viral complications due to primary infection or reactivation (e.g., by cytomegalovirus (CMV), BK polyomavirus (BKPyV), or Epstein Barr virus (EBV)). After kidney transplantation, BKPyV primary infections or reactivations can lead to BKPyV-associated nephropathy (BKPyVAN) with renal malfunction and risk of graft loss [1–3]. The BKPyV infection is widespread with a seroprevalence of more than 80% in adults [4, 5]. After primary infection (mainly during childhood), BKPyV persists in the renourinary tract [6]. While BKPyV infections in healthy individuals generally take an asymptomatic course, they are a major cause of graft dysfunction after kidney transplantation with up to 10% of all kidney transplant recipients developing a BKPyVAN that results in allograft loss in 10–80% [1, 7–10]. Male gender and pediatric or older recipients have been identified as independent risk factors for BKPyV viremia after

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kidney transplantation [10–12]. Hoecker et al. have just shown that uncontrolled BKPyV replication affects a significant proportion of pediatric renal transplant recipients being associated with unique features of epidemiology and risk factors such as young recipient age, obstructive uropathy, and overall intensity of immunosuppressive therapy [13]. The increase in the incidence of BKPyVAN after kidney transplantation was clearly associated with the introduction of new and more potent immunosuppressive drugs successfully used for the prevention and treatment of acute rejection. A significantly increased risk of BKPyVAN was reported for tacrolimus (TAC) compared to cyclosporine A (CsA)-based regimens [11, 12, 14], while a reduced risk of BKPyV viremia and BKPyVAN was associated with mammalian target of rapamycin (mTOR) inhibitor-containing therapies [11, 15–18].

Based on a survey among pediatric transplant centers, diagnosis and treatment of BKPyV-infections vary considerably between different countries [19].

Diagnosis

Considering current knowledge of development and progression of BKPyVAN, the importance of early diagnosis must be highlighted [1]. Some years ago, the detection of “decoy cells” in urinary cytology has been the first diagnostic test for BKPyV infection/reactivation and BKPyVAN. Unfortunately, the positive predictive value for BKPyVAN is only 5–29% and intra-observer variability is high [2, 10, 20]. Currently, the fundament of BKPyV diagnostics is the detection of BKPyV-DNA via polymerase chain reaction (PCR). Primarily, BKPyV-PCR has been performed in the urine as this material is easily accessible. The number of copies of DNA-PCR in urine is associated with a low positive (31%) but good negative (100%) predictive value and can therefore be used for screening followed by BKPyV-PCR in the plasma in case of positive results [20]. But as this strategy may double the costs and might cause a diagnostic delay, a primary measurement of the BKPyV viral load in plasma can be recommended. It has been proven that BKPyV viremia is associated with the risk of development of BKPyVAN [21]. In 2002, Hirsch et al. showed that mean viral load in plasma was significantly higher in patients with biopsy-proven BKPyVAN than in patients without BKPyVAN [2]. For BKPyV viremia (> 1.600 copies/mL), Viscount et al. calculated a sensitivity of 100% for nephropathy and a positive predictive value of 50% [20]. Ginevri et al. have found a longer duration of viremia in patients with higher peak plasma loads [22]. Therefore, regular screening for BKPyV replication by plasma viral load is recommended in kidney transplant recipients [23]. During the first 6 months after transplantation, monthly plasma screening is suggested, with decreasing frequency thereafter [24, 25]. In a single-center study, Schachtner and colleagues

analyzed 103 adult kidney recipients with BKPyV viremia between 2004 and 2012. The highest incidence of BKPyV viremia was observed in the early post-transplant period (< 6 months after transplantation) with 65% of cases; the median time of diagnosis was 4 months after kidney transplantation [26]. In our own pediatric study of 31 kidney recipients, the highest incidence of viremia was also found during the initial post-transplant period, but the median time to first positive testing of blood BKPyV-DNA was 1.9 months after transplantation (range 0.3–35.9 months) and 77% of patients showed first BKPyV positivity within 6 months after transplantation (data not published). The shorter median time to first positivity in our pediatric study group may have been because of younger recipient age, with the resulting higher proportion of primary BKPyV infections based on an increased incidence of high-risk constellations. Where relevant BKPyV viremia is detected, a standardized graft biopsy according to BANFF guidelines [27] should be performed using immunohistochemistry (SV40 T antigen staining) or in situ hybridization to prove the presence of BKPyVAN (Fig. 1). However, negative biopsy results do not necessarily rule out early focal BKPyVAN since the probability of sampling error amounts to at least 10–36.5% of cases due to the focal nature of BKPyVAN [28]. Accordingly, in patients with sustained BKPyV viremia ($> 4 \log_{10}$ copies/mL), a diagnosis of “presumptive BKPyVAN” should be made in the case of an absent or negative kidney biopsy [23].

Treatment

There is no standardized treatment algorithm for post-transplant BKPyV infections. Intensity of immunosuppressive treatment after kidney transplantation seems to be the key issue in the pathogenesis of BKPyV replication. Therefore,

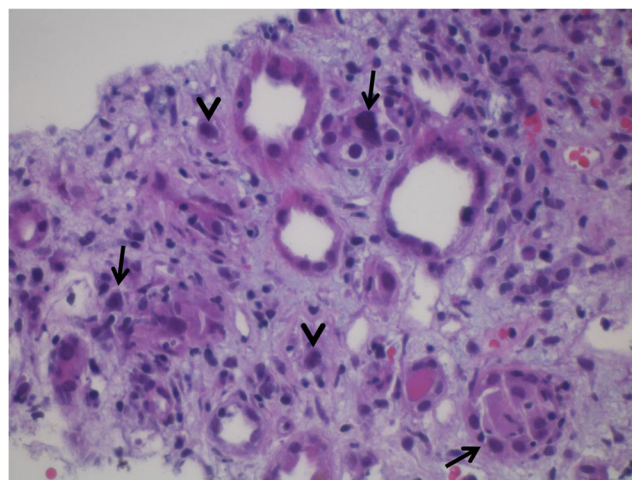


Fig. 1 BKPyVAN: numerous tubular epithelial cells show enlarged chromatin-rich nuclei (arrows), some of these cells in the lumen (decoy cells, arrowhead)

Table 1 Adjuvant antiviral strategies for the treatment of BKPyV replication

Drug	Possible mode of activity*	Toxicities/side effects
Cidofovir [24, 29–34]	Nucleoside analog of cytosine (HPMPC) with antiviral activity: inhibition of DNA polymerase*	Nephrotoxicity (up to renal failure), myelosuppression (leucopenia), nausea, diarrhea, uveitis/iritis, ocular hypotonia, alopecia, skin eruption, headache, fever, asthenia
Leflunomide [24, 33–38]	Malononitrilamide with immunosuppressive and antiviral activity: inhibition of pyrimidin synthesis and protein kinase activity; inhibition of virion assembly by preventing tegument acquisition by viral nucleocapsids*	Myelosuppression (leucopenia), liver toxicity (elevation of liver enzymes), CK-elevation, colitis, nausea, diarrhea, hypertension, skin eruption, alopecia, tendinitis, peripheral neuropathy, headache, inappetence, asthenia
Fluoroquinolones [24, 33, 34, 36, 39, 40]	Quinolone antibiotics with antibacterial and antiviral activity: inhibition of topoisomerase II (bacterial DNA gyrase) and topoisomerase IV; inhibition of helicase of large T antigen*	Nausea, diarrhea, leucopenia, myalgia, arthralgia, tendinitis
IVIg [24, 33, 41]	Substitution of human antibodies (IgG): anti-inflammatory immunomodulation; direct neutralizing activity by anti-virus antibodies*	Allergic reactions

*The exact mechanism by which all these agents mediate anti-BKPyV activity is still unclear.

reducing immunosuppression has become current practice in most transplant centers.

Antiviral therapies such as cidofovir [24, 29–34], leflunomide [24, 33–38], fluoroquinolones [24, 33, 34, 36, 39, 40], and intravenous immunoglobulin (IVIg) [24, 33, 41] are also considered. Some in vitro studies showed inhibition of BKPyV DNA replication by these antiviral strategies [24, 32–34, 40, 42], but randomized controlled studies in vivo are missing. The published case series provided contradictory results [24, 29–31, 33–39, 41]. While some authors have not found a demonstrable benefit, other case series have reported a favorable outcome of these antiviral strategies with stabilization of renal function and/or clearance of viremia. However, since immunosuppression had been reduced or discontinued prior to or concomitantly with administration of cidofovir, leflunomide, fluoroquinolones, or IVIg in these cases, the effect of these agents in addition to reduced immunosuppression is still not resolved and awaits a randomized study with appropriate statistical power [24, 33]. Even though there are no randomized controlled trials providing evidence that adjunctive use of these agents is superior to timely reduction of immunosuppression, the current guidelines suggest that the “adjunctive use of antiviral agents may be considered” in patients with sustained high-level plasma BKPyV load despite reduction of immunosuppression [24]. In contrast to cidofovir, leflunomide, and IVIg, fluoroquinolones may even only be associated with some prophylactic efficacy [24, 39]. Table 1 gives an overview on the mechanisms and toxicities of these drugs.

In addition, several studies have previously shown that not only the dosage but also the type of immunosuppressive drug significantly influences the risk of BKPyVAN [11, 15, 43]. This observation is in accordance with several publications indicating that TAC-based immunosuppression is associated with an increased risk of BKPyV viremia and BKPyVAN [10–12, 18, 24, 44, 45]. The role of a switch to mTOR

inhibitor-based regimens is still a matter of debate [15, 19, 45, 46]. In recently published studies, mTOR inhibitor-containing therapies showed a reduced incidence of BKPyV viremia and BKPyVAN after kidney transplantation, especially in combination with low-dose CsA as opposed to TAC [11, 15–18]. In this context, Hirsch et al. recently showed that BKPyV replications in renal tubular epithelial cells could be inhibited by CsA and the mTOR inhibitor sirolimus, but activated by TAC [46]. This experimental data confirmed our clinical observations that de novo therapy with low-dose CsA and everolimus seems to be superior concerning outcome of BKPyV infections after pediatric kidney transplantation (Ahlenstiel-Grunow et al. submitted). Based on these observations, pre-emptive reduction and modification of maintenance immunosuppression (e.g., switch to CsA and/or mTOR inhibitor) is currently recommended [23] for patients with presumptive or biopsy-proven BKPyVAN to regain immunologic control [1, 23, 25]. Table 2 describes a possible therapeutic algorithm for BKPyV based on this data.

In recent years, it has become clear that early diagnosis combined with timely pre-emptive reduction of immunosuppression is crucial for the outcome of BKPyVAN [1]. The prognostic importance of early diagnosis was highlighted by the observation that early biopsy-proven diagnosis of BKPyVAN followed by therapeutic intervention at time of stable renal function resulted in an improved outcome compared to late biopsy-proven BKPyVAN at time of graft dysfunction [47, 48]. Several prospective single-center studies subsequently showed that preemptive reduction of immunosuppression in the case of BKPyV viremia is an effective strategy to achieve clearance of viremia and prevent onset of BKPyVAN in 80–100% of renal transplant recipients [14, 22, 43, 44], whereas therapeutic intervention at a late stage of allograft involvement has shown only a marginal beneficial effect [8, 49]. However, this strategy of pre-emptive reduction of immunosuppression might increase the risk of inducing

Table 2 Possible therapeutic algorithm for BKPyV-associated nephropathy

1st step
Dose reduction of immunosuppressive therapy as calcineurin inhibitors and/or mycophenolate
2nd step
Switch from tacrolimus to low-dose cyclosporine A
and/or switch to mTOR-inhibitor plus low-dose calcineurin inhibitor
3rd step
Cessation of calcineurin inhibitor
4th step
Consider adjunctive treatment with cidofovir (nephrotoxicity!), leflunomide, and/or immunoglobulin (efficacy not proven)
Consider results of BKPyV-PCR and levels of BKPyV-specific T cells during reduction and re-increase of immunosuppressive therapy

acute graft rejection, so kidney function has to be monitored in close intervals and in worse case scenarios. BKPyVAN is associated with acute rejection after reduction of immunosuppressive therapy. Of note, several studies have described kidney recipients with rapid, self-limited BKPyV viremia without therapeutic intervention [3, 50, 51]. This illustrates that guidance by viral load only may lead to unnecessary reduction of immunosuppressive therapy with the attendant risk of under-immunosuppression.

Immune response

The pathophysiology of BKPyVAN is complex and the level of BKPyV-DNA in plasma alone is insufficient to estimate the risk of onset of BKPyVAN and to decide upon necessity of therapeutic intervention [44]. It is known that BKPyV viremia after kidney transplantation does not result inevitably in BKPyVAN. Many kidney recipients show self-limiting BKPyV viremia without therapeutic interventions [3, 50, 51]. In these cases, pre-emptive reduction of immunosuppression is not only unnecessary but also associated with an increased risk of rejection. Elfadawy et al. have demonstrated that patients with persistent high viremia (> 10.000 copies/mL) had an increased risk for BKPyVAN, whereas transient high viremia had no such association [3]. Moreover, our own pediatric study has not found any correlation between level of BKPyV-DNA load and subsequent duration of viremia (Ahlenstiel-Grunow et al. submitted). Accordingly, detection of BKPyV viremia is a useful screening parameter, but not suitable for predicting the individual course of BKPyV infections and the risk of BKPyVAN. Therefore, reliable parameters are urgently needed to distinguish patients with self-limiting, short-term viremia from those with long-term viremia, and thereby to prevent unnecessary reduction of immunosuppression.

In the search for suitable predictive parameters, BKPyV-specific humoral responses have not proven beneficial and do not appear to play a major role in containment of BKPyV

infections [52]. It was found that a rise in BKPyV-specific IgG levels is strongly associated with active viral replication. However, this increase could be detected during as well as after BKPyV viremia [22, 53–55]. Recently, the studies of Schachtner et al. and Leboeuf et al. reported no correlation of BKPyV antibody levels with plasma BKPyV-DNA load and viral clearance [51, 56]. Accordingly, BKPyV antibodies have no prognostic value because the detection of BKPyV antibodies is combined with BKPyV viremia regardless from subsequent clinical course of the BKPyV infection.

In contrast to BKPyV antibodies, virus-specific T cells (Tvis) have been shown to play an important role in controlling viral replication of latent viruses such as CMV, EBV, and BKPyV [57]. Tvis measurements can be performed by different methods. In contrast to enzyme-linked immunospot (ELISpot), flow cytometry permits the differentiation between CD4 and CD8 T cells. Several groups have already proven the potential of CMV-Tvis as a prognostic marker for the clinical course of CMV-infections [57–59]. Concerning BKPyV infections, a few adult studies recently observed that an increase of BKPyV-specific cellular immunity coincided with viral clearance in kidney transplant recipients [60, 61]. Accordingly, an insufficient BKPyV-Tvis level seems to be a key mechanism of BKPyV-associated complications after kidney transplantation. The pediatric trail by Ginevri et al. monitored BKPyV-specific T cells in 13 pediatric viremic kidney recipients under reduced immunosuppression and confirmed that a reduction of plasma-BKPyV-DNA is associated with an increase in BKPyV-Tvis supporting the theory that the expansion of specific immunity to BKPyV has a protective role [22]. Concerning BKPyV reactivations, Costa et al. observed episodes of BKPyV reactivation only in patients without a BKPyV-specific cellular immune response [62] and Schachtner et al. recently demonstrated that kidney transplant recipients with loss of BKPyV-Tvis over the pre- to post-transplant period were at increased risk of BKPyV replication [63]. In 2011 and 2014, Schachtner et al. reported in a small study group of viremic patients that kidney recipients with self-limited BKV reactivation developed BKPyV-Tvis

without therapeutic intervention, whereas patients with BKPyVAN showed BKPyV-Tvis only after therapeutic interventions [50, 51]. Moreover, our own monocentric prospective, longitudinal study including 33 viremic children after kidney transplantation revealed the prognostic value of BKPyV-Tvis and demonstrated a negative correlation between number of BKPyV-Tvis and subsequent duration of viremia after kidney transplantation: High BKPyV-CD4 and/or CD8 Tvis predict asymptomatic BKPyV infections with self-limiting, short-term viremia (< 120 days), whereas lack or low levels of BKPyV-CD4 Tvis were associated with long-term viremia and florid BKPyVAN (Ahlenstiel-Grunow et al. submitted). Of note, BKPyV-Tvis level correlated with the subsequent duration of viremia but not with the BKPyV-DNA load in plasma, highlighting the additional benefit of BKPyV-CD4Tvis. The detection of BKPyV-CD4 Tvis (> 0.7 cells/ μ L) and/or CD8 Tvis (> 0.5 cells/ μ L) revealed a positive predictive value of 0.96 and a negative predictive value of 0.75 for self-limiting viremia. After minimization of immunosuppressive therapy and/or switch to mTOR inhibitors BKPyV-CD4 Tvis increased with subsequent decrease of plasma BKPyV-DNA (Ahlenstiel-Grunow et al. submitted).

These data highlighted the predictive value of BKPyV-Tvis after pediatric kidney transplantation to distinguish patients with self-limiting, short-term viremia from those with long-term viremia. Serving as a prognostic marker, BKPyV-Tvis may therefore identify patients at risk of BKPyVAN and thereby individualize therapeutic interventions. Based on these data, we suggest a possible diagnostic algorithm for BKPyV infections including Tvis measurements as described in Fig. 2.

Conclusion

New diagnostic strategies using markers of the individual cellular immune response seem to be promising in pediatric kidney transplantation to estimate the outcome of BKPyV infections, avoid unnecessary pre-emptive reduction of immunosuppression, and thereby reduce the risk of acute rejections. In Fig. 2 and Table 2, we present possible diagnostic and therapeutic algorithms for BKPyV. The measurement of BKPyV-Tvis at time of onset of BKPyV viremia with the question, whether a reduction or change of immunosuppressive therapy should be performed, could become a part of routine care. Prospective, interventional trials comparing standard of care with Tvis-based steering of immunosuppressive therapy are needed in order to confirm this strategy in viremic patients. In such a future study, a pre-emptive therapeutic intervention should only be performed in case of insufficient BKPyV-specific cellular immune response, and the reduction of immunosuppression and/or the switch to mTOR inhibitor-based regimen should be guided by BKPyV-Tvis level. Regarding the value of BKPyV-Tvis for successful control of virus replication, a future therapeutic approach may include an early infusion of autologous, MHC-restricted, BKPyV-Tvis to counterbalance the increased risk of BKPyVAN conferred by insufficient development of BKPyV-specific immunity.

Questions (Answers are provided following the reference list)

1. What is no diagnostic mean for detection of BKPyV replication with risk of nephropathy?
 - A) Decoy cells in the urine
 - B) BKPyV PCR in urine
 - C) BKPyV PCR in blood
 - D) Graft biopsy with SV40 staining
 - E) BKPyV-specific IgG in blood
2. Name the current recommendation for first-line treatment of BKPyV associated nephropathy
 - A) Reduction of immunosuppressive therapy
 - B) Switch of immunosuppressive medication to Belatacept-based immunosuppression
 - C) Cidofovir

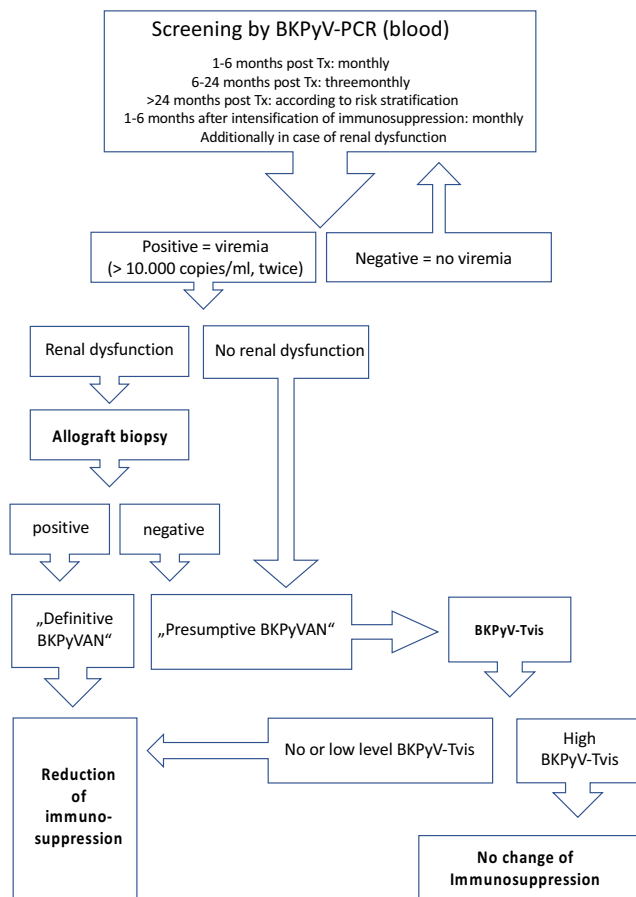


Fig. 2 Suggested diagnostic algorithm for BKPyV infections including measurements of BKPyV-specific T cells (BKPyV-Tvis)

- D) Ciprofloxacin
E) Leflunomide
3. What is NOT true? BKPyV-specific T cells
- A) represent the cellular immune response to BKPyV.
B) predict the ability of the transplanted patient to cope with BKPyV infection.
C) have the same predictive value as BKPyV-specific antibodies.
D) can be determined by ELISpot-assay or flow cytometry.
E) have not yet been implemented in routine care.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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Answers:1. E; 2. A; 3. C