Review

Polyomavirus BK in Renal Transplantation: Virological Notes for Monitoring and Diagnosis

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Review

Polyomavirus BK in Renal Transplantation: Virological Notes for Monitoring and Diagnosis

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Abstract

Polyomavirus-associated nephropathy was first reported more than 50 years ago, however it still represents a cause of renal injury, with occurrence rates of 1%-10%, particularly in the firs two years following kidney transplantation. The role played by immunesuppression in viral reactivation is well aknowledged and the modulation of immunesuppression level is the main strategy for the management. Viral and immunological evaluation are fundamental for optimizing the diagnostic and clinical-therapeutic pathway. In this review, main features of polyomavirus BK and associated nephropathy will be addressed from a virological point of view.

Keywords: polyomavirus BKV; polyomavirus-associated nephropathy; renal transplantation

1. Polyomaviridae Family

At present, fifteen human polyomaviruses have been described (Table 1), in addition to two primate viruses also found in humans, i.e. SV-40 (in 1960s) and B-lymphotropic PyV (in 2010). The first polyomavirus was isolated in 1953 by Ludwig Gross, i.e. murine polyomavirus (MPyV). Given the ability of inducing tumor development, this virus was given the name "polyomavirus" (from the greek: poly = many and oma = tumor), and it was observed to cause adenocarcinoma of the parotid gland in newborn mice. In 1971, human polyomaviruses BK and JC were first reported in urine from a kidney transplant patient with ureteral stenosis and from postmortem brain tissue of a patient with progressive multifocal leukoencephalopathy, respectively; these viruses were named after the initials of the two patients. Nevertheless, in 1980's up to late 90's, polyomavirus-associated nephropathy was still neglected as specific disease to be considered in the context of kidney transplantation, rather focusing on the management and prevention of graft rejection. It was only at the end of past century that BK virus evaluation and monitoring in kidney transplantation started, although always in the absence of specific indications on therapy. From 2007 up to now, other polyomaviruses have been isolated, mainly in subjects with immunesuppressive conditions, although in many cases with no specific disease association. Of these viruses, eight have been found on healthy skin and are actually considered as part of the human cutaneous virome. Some of these viruses are not linked to specific dis

Table 1. Polyomaviruses of interest in human pathologies. Two other primate viruses have been also reported in humans. IS, immunesuppression; PML, progressive multifocal leukoencephalopathy; IC, immunecompetence; TS, trichodysplasia spinulosa.

VIRUS	HUMAN PATHOLOGIES	IMMUNE STATUS	FIRST DESCRIPTION
BK	Nephropathy, heamorrhagic cystitis	IS	1974 Urine, kidney tranplant pt

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JC	PML	IS	1974 Postmortem brain, PML
KI	Mild infection of respiratory tract?	IC+IS	2007
WU	Mild infection of respiratory tract?	IC+IS	2007
Merkel cell PyV	Merkel cell carcinoma	IC+IS	2008
HPyV6	Epithelial proliferation, itching, discheratotic dermatitis (sporadic), keratoacanthoma?	IS	2010
HPyV7	Epithelial proliferation, itching, discheratotic dermatitis (spo-radic), thymoma?	IS	2010
Trichodysplasia spinulosa pyV	TS	IS	2010
HPyV9	Hyperkeratotic papules and plaques (sporadic)	IS	2011
HPyV10 and variants MWPyV,MXPyV	-	-	2012
Saint-Louis PyV	Infant diarhrea?	-	2013
HPyV12	-	-	2013
New Jersey PyV13	Myositis, cutaneous necrosis,, vasculitis (1 case)	IS	2014
Lyon IARC PyV	Infant diarhrea?	-	2017
Quebec PyV	-	-	2019

eases and could be expression of transient infection, environmental exposure or viral contamination [1]. The most pathologically important cutaneous polyomavirus MCPyV causes the majority of cases of Merkell Cell carcinoma, a rare, but highky aggressive malignant neoplasm occurring in both immunocompromised and immunocompetent individuals. Moreover, primary infection with Trichodysplasia Spinulosa-associated Polyomavirus (TSPyV) is associated to the very rare occurrence of this disease in immunocompromised subjects [2–16]. The ubiquitous occurrence of polyomaviruses in human population has been reported in a study on blood donors in which antibodies against at least four polyomaviruses were found in all the individuals, and the seroprevalence ranged from 60% to 100% [17].

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2. Polyomavirus BK (BKV) and Polyomavirus-Associated Nephropathy

BKV was first isolated in 1971 [2]; however, at that time, its pathogenic role remained elusive and BKV was considered an orphan virus for many years afterwards.

In 1978 and subsequent years, main features of nephropathy in kidney transplantion were first described, including the detection of urine decoy cells, the presence of viral inclusions in uroepithelial cells in graft biopsies, the challenging differential diagnosis with acute rejection, and the role of immunosuppression in the development of renal damage. Nevertheless, at that time, polyomavirus-associated nephropathy was not already recognized as a specific entity [18–21].

In 1995, Purighalla and coll. first described a case of PVAN, thus recognizing it as a definite disease entity [22]. Subsequently, several reports from many transplant centers worldwide with increasing prevalence rates were published.

Although epidemiology of PVAN may vary in different transplant centers, incidence has been reported in 1-10% of kidney transplant recipients, mainly in the first two years following transplantation. PVAN may lead to graft loss and return in hemodialysis in 30-80% of the cases within 6-60 months [23,24], with a significant impact on the quality of life and the outcome of transplant procedure.

BKV is the etiological agent in most of the cases, whereas JCV has been rarely (<1-3%) associated to PVAN, either alone or in association to BKV [25–32].

BKV belongs to the genus Betapolyomavirus of the *Polyomaviridae* family and is a small, non-enveloped, icosahedral and double-stranded virus, with a diameter of about 40-44 nm and a genome consisting of approximately 5000 base pairs. The genome is constituted by three regions: the early and the late coding regions and the non-coding control region (NCCR). The early coding region encodes for regulatory proteins in viral replication and transcription: large T antigen (AgT) and small t antigen (Agt); these genes are transcripted before viral DNA replication. The late coding region encodes for structural proteins VP1, VP2, VP3 and the non-structural agnoprotein. Whereas early and late coding regions are highly conserved, the NCCR sequence displays high variability with the possible emergence of mutant strains that may determine changes in the cell tropism, viral fitness, potential of replication, features of pathogenicity and virulence. The VP1 protein also exhibits high genetic variability; based on single nucleotide polymorphisms in VP1 and NCCR, BKV can be divided into four main genotypes: I-IV. Type I is the prevalently circulating strain and accounts for the maiority of BKV-related diseases. Type I and type IV can be further divided into four (Ia, Ib-1, Ib-2, Ic) and six (IVa-1, IVa-2, IVb-1, IVb-2, IVc-1, IVc-2) subtypes. Globally, type I accounts for approximately 80% of viral isolates and type IV for approximately 15%, whereas type II and III are poorly represented. Molecular variants (genotypes and subtypes) present differences in terms of geographical distribution. For instance, subtype Ia is the most frequent in Africa, Ib1 in southeast Asia, Ib-2 is most common in Europe, whereas Ic is the most frequent in Northeast Asia. Genotypes may present different biological behaviour determining also differences from a clinical point of view; for example, type I strains have higher growth in human uroepithelial cells [33].

BKV is ubiquitously distributed in the population with a seroprevalence rate up to 90% by the age of four [13]; primary infection usually occurs early in the childhood, usually by mucosal contact in the oral and respiratory tracts, and is often asymptomatic or presenting only mild upper respiratory tract manifestations. Subsequently, BKV can establish a lifelong latent infection in uroepithelial cells as the most relevant latency site, as well as more rarely in other cell types (e.g. endothelial cells, fibroblasts, B cells, brain). Immunocompetent individuals usually exhibit no significant clinical symptoms throughout their lives, with the possible exception of transient viruria. In immunocompromised patients, such as solid organ transplant recipients, hematopoietic stem cell transplant recipients, and AIDS patients, BKV may reactivate because of the impairment of cell-mediated immune response. In particular, in kidney transplant patients reactivation manifests as sequential occurrences of viruria and viremia. Tubular epithelial cells are the main target with the onset of tubulointerstitial nephritis. Polyomavirus-associated nephropathy usually occurs in the first two years following renal transplantation. Nephropathy of native kidney of other solid organ

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transplant recipients has been also described, but is very infrequent. In stem cell transplant recipients, BKV reactivation may be associated to hemorrhagic cystitis or hematuria with interstitial nephritis. The *condicio sine qua non* in the onset of viral reactivation and consequent disease is the immunesuppression; in particular, it is likely that the overall intensity of immunesuppression, rather than a specific anti-rejection agent, is the key factor. The incidence of BK viruria and viremia in kidney transplantation is reported to be approximately 30 % and 12 %, respectively [1], with viremia usually occurring within 2 to 6 weeks after the onset of viruria, and approximately half of viremic patients progress to PVAN.

Although there are no vaccines or antiviral agents established for the treatment of PVAN, clinical management may benefit of significant advances and ameliorations obtained in recent years in terms of monitoring strategies, diagnostics, and therapeutic approaches.

3. Strategies for Diagnosing, Monitoring, and Managing Polyomavirus-Associated Nephropathy

Recently, the American Society of Transplantation Infectious Diseases Community of Practice has published guidelines for BKV in solid organ transplantation [34] and The Transplantation Society International BK Polyomavirus Consensus Group has updated recommendations by publishing in 2024 the Second International Consensus Guidelines on the Management of BK Polyomavirus in Kidney Transplantation [35].

Particular attention in considering the strategies for monitoring and managing PVAN is given to risk factors associated to the onset; these are usually considered in terms of donor, recipient, and transplantation. Among donor factors, donor viruria (urinary shedding of BKV), high anti-BKV-VP1 antibody levels, certain BKV genotypes as well as mismatching between donor and recipient genotypes are considered. Recipient-related risk factors for BKV viremia include older age and male gender, seronegativity for anti-BKV-VP1 antibodies, previous kidney transplantation, low recipient neutralizing antibodies levels against donor BKV serotype and the absence of certain potentially protective HLA types (such as A2, A24, B7, B8, B13, B44, B51, Cw7, and DR15); in pediatric transplantation, also younger recipient age and obstructive uropathy as primary renal disease represent risk determinants. Considering factors related to the transplantation, a higher risk of viremia seems to be associated with the use of tacrolimus compared with cyclosporine, T cell–depleting agents, higher corticosteroid use, acute rejection events, AB0-incompatible transplants, and ureteric stents. Eder and coll. [36] have recently performed an extensive meta-analysis of 165 publications, including prospective and retrospective studies, encompassing more than 197,000 patients. The authors evaluated modifiable risk factors in relation to main endpoints, including biopsy-proven PVAN, presumptive PVAN, detection of viremia, and infection requiring treatment and concluded that no single independent risk factor for all endpoints was identifiable, thus underlying the complexity in pathogenesis and in predicting BKV-associated impact and complications in renal transplant recipients. It is likely than the overall net immune level, rather than a single drug or determinant, concur to the onset of nephropathy.

According to guidelines, a possible diagnosis of BKV nephropathy is made in the presence of urine viral load >10 million genome copies/mL; a probable diagnosis in the presence of BKV DNaemia >1000 genome copies/mL for at least two weeks; whereas a presumptive diagnosis is made for an increasing viremia >100000. Biopsy-proven BKV-nephropathy is diagnosed on the basis of the detection of suggestive cytopathic effects plus immunohistochemistry and a specific diagnostic test for BKV in differentiation to JCV [34,35,37].

As the impairment in immune response is the condicio sine qua non for the development of PVAN and given the fact that there is no effective antiviral agent to prevent or treat BKV replication, the milestone for clinical-therapeutic management of sustained BKV-DNAemia or biopsy-proven nephropathy in kidney transplant patients relies on the modulation of immunesuppression with the aim of obtaining a sufficient BKV-specific immune control and avoid rejection episodes. Taking into account this, early evidence of viral reactivation is fundamental for a “pre-emptive” reduction of

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immunesuppression and BKV monitoring should be focused on viral replication and, potentially, cellular immune response evaluation.

The knowledge of biological behavior and clinical course of viral reactivation and nephropathy are the key points for establishing a monitoring strategy. Viral reactivation usually starts with the appearance of persistent asymptomatic viruria (>10^6 copies/mL) or the occurrence of decoy cells in urine. These are virally infected uroepithelial cells, observed in urine cytology of kidney transplant recipients; decoy cells are characteristically identified by their enlarged nuclei, intranuclear inclusions, and other morphological changes indicative of viral infection. It is to note that, although decoy cells can be used to screen for BKV infection, they are not always specific for it and may be present in other conditions, thus exhibiting an overall low sensitivity [38,39]. Viruria is typically followed by viremia within two to six weeks. The finding of persistent viremia (>10^{4-5} copies/mL for at least three consecutive weeks) is usually indicative of uncontrolled viral replication, thus leading to tissue damage. Viruria and viremia usually precede the increase in serum creatinine. Despite the availability of these surrogate markers of viral replication, the definitive diagnosis of PVAN is made by histopathology. The sensitivity of histological diagnosis is directly proportional to the time from the occurring of viremia and is related to viral load (both in urine and plasma). A negative histology is relatively frequent in the initial stages of PVAN [40], as well as it may be challenging in consideration of the multifocal nature of the disease, with possible false negative results. Although not routinely performed, also quantitative evaluation of BKV DNA on kidney biopsy by molecular assay could be performed, with high viral loads (>10^4 genome equivalents/cell) being associated to histopathologically confirmed PVAN [41]. Options for management are related to the stage of progression of the disease. In patients with viruria and viremia, a preemptive reduction of immunesuppression, before the occurrence of tissue damage, may lead to resolution in 85%-90% of the cases with maintenance of renal function. Subsequently, in case of late diagnosis with already established tissue damage, resolution is less probable and the rate of early loss of the graft rises from less than 10% up to 30%. In endstage PVAN, treatment is usually late and inefficacious with progressive obliteration of renal tubula (primary target of BKV infection) and progressive decrease of viruria and viremia.

According to Consensus Recommendations [35], screening of viral replication allows for identification of patients at risk, with a negative predictive value of 100% (i.e. no replication = no PVAN) and evaluation of temporal profile of replication. Overall, this allows for a timely management and the subsequent monitoring of response to the treatment (modulation of immunesuppression), as supported by several studies [42–44].

Screening for BKV replication is regularly recommended in renal transplant recipients and consists into the quantification of BKV-DNA on plasma specimen monthly up to 9 months posttransplantation; subsequently every three months up to two years or in the occurrence of events, such as graft dysfunction or renal biopsy (performed for surveillance or upon clinical indication: suspected rejection or nephropathy). In case of persistent BKV-DNA on plasma (>10^4 copies/mL), viremia should be re-evaluated within 2-3 weeks and monitoring should be continued at 2-4 weeks intervals (Table 2). In case of combined renal and other solid organ transplantation, screening should be performed every three months up to three years posttransplantation. In other solid organ transplantation, screening for BKV is not indicated, it could be considered in the occurrence of renal dysfunction. In this case, if renal biopsy is taken, PVAN should be evaluated.

As regards the potential usefulness of kidney biospy for diagnostics, definitive diagnosis is made by histological analysis. Studies have not evidenced a relation between histopathological staging and the level of viral replication (BKV DNAemia); for instance, we previously reported a wide range of viremia (from 10^4 to 10^6 copies/mL) in biospy-proven nephropathy [45,46]. According to our Center practice, monitoring for PVAN is actually made by evaluation of BKV DNA on plasma specimen as recommended by International Guidelines. In the presence of persistent viral load >10^4 copies/mL in addition to abnormalities in renal function, a presumptive diagnosis of PVAN is made. Biopsy is than performed in order to make a definitive diagnosis and stage the nephropathy. Monitoring of BKV

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DNAemia is therefore performed in order to evaluate the response to the reduction of immunesuppression, whereas biospy is performed to evaluate the course of histological damage.

From the laboratory point of view, some technical factors should be taken into account. First, monitoring of BKV-DNA viremia should be performed on the same type of specimen and with the same molecular assay for a reliable monitoring along the time. Quantitative evaluation of viremia is superior to cytology, particularly in terms of sensitivity and positive predictive value. Molecular assays should target conserved genome sequences in order to detect all genotypes and subtypes and short amplicon (less than 150 base pairs)

Table 2. Screening of Polyomavirus BK in transplant recipients, according to the Second International Consensus Guidelines on the Management of BK Polyomavirus in Kidney Transplantation [35].

KIDNEY TRANSPLANTATION	BKV-DNA QUANTITATION ON PLASMA Monthly up to month 9 Every three months up from month 10 to month 24 Monthly for three months in the occurrence of graft dysfunction or biopsy performed for surveillance or upon clinical suspicion (rejection, nephropathy) In case of persistent viremia (>10^{3-4} copies/mL), confirmation within 2-3 weeks and monitoring every 2-4 weeks
KIDNEY COMBINED TRANSPLANTATION	BKV-DNA QUANTITATION ON PLASMA Screening extension up to month 36
OTHER SOLID ORGAN TRANSPLANTATION	Screening not indicated, consider in the occurrence of renal dysfunction

should be used to avoid subquantification. As regards BKV DNA quantification, as significant interassay variability precludes the appropriate use of thresholds for the management of BKV infection in transplantation, the 1st WHO International Standard for BKV (primary standard) was introduced in 2016 (First WHO international standard for BK virus DNA. NIBSC code, 14/212. WHO, Geneva, Switzerland). However, subsequent studies have evidenced that the WHO standard contains subpopulations of viruses with mutations in the T region that may determine molecular result variations depending on which region of the WHO standard is targeted [47]. Therefore, the need for developing commutable international standards for BKV-DNA loads (plasma, whole blood, urine, and tissue) based on defined molecular sequences still subsists. Another factor that should be considered is the clinical importance of a negative assay. On the other hand, it should be taken into account that viral replications does not always mean nephropathy and cases of high level and persistent viral replications have been reported in the absence of kidney abnormalities in transplant recipients [48].

As previously mentioned, most cases of PVAN are associated to BKV, however concurrent JCV-DNAemia has been reported and correlated with poor graft outcome in kidney transplant recipients with nephropathy [49]. In the study by Zhang et coll., concomitant positivity for JCV viremia was evaluated in a population of 140 kidney tranplant patients and was found in 18 individuals. In this study, the graft loss rate in the JCV-DNAemia positive group was significantly higher than in the negative group. The authors concluded that JCV-DNAemia was an independent risk factor for graft survival with an odds ratio of 4.808.

Given the role of immunesuppression in the pathogenesis of PVAN, as well as the potential therapeutic strategies, also BKV-specific immunity plays a relevant role in the diagnostic pathway of nephropathy, including both the humoral and cellular arms. As regards humoral response,

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standardized and validated serological assays in the transplant setting are lacking, as well as no distinction between serotypes is available (i.e. potentially useful for risk stratification, allocation, screening and personalized treatment). A clear role for humoral evaluation in the management of kidney transplant patients is not yet defined. However, some studies have evidenced that in seropositive donors, the risk of viremia and nephropathy are higher, particularly in the presence of high levels of IgG with low or undetectable levels in the recipient. Similarly, seropositive recipients before transplantation are not protected toward viral replication and PVAN, although a certain level of neutralizing antibodies against the donor specific serotype is related to a lower number of posttransplantation episodes of BKV DNAemia [50–54]. As regards humoral immunity, some unmet needs subsist, including the availability of standardized and validated serological assays in the transplant setting; the lack of distinction between serotypes, that would be necessary for risk stratification, organ allocation, screening and tailored treatment.

Considering cellular immunity, this seems to play a role in the course of viral replication. Healthy seropositive subjects present CD4+ and CD8+ T cells that are specific for Tag and VP1 and reduction of immunesuppression is associated to the development and reconstitution of cell mediated immunity and the clearance of BKV DNAemia [55,56]. Nevertheless, cell mediated immunity evaluation is difficult to be performed, given the difference between methods, antigens used for stimulation, operating features, timepoints of evaluation, and more other factors. Also in this case, some unmet needs subsist, including the lack of standardized and clinically validated assays to guide immunesuppression and the application of adoptive transfer of virus-specific T cells. In a small study perfomed by our group by using an internally developed Elispot assay with an antigenic stimulus obtained with a peptide mix of sequences of VP1 and LargeT antigen, out of 149 renal transplant patients, less than 10% resulted responders in the first year following transplantation, with no case of viral reactivation (positivity to viremia and/or viruria) in responders [57].

Retransplantation may represents an option in selected cases. Previously published data reported a three-year survival rate higher than 96%, with an overall rate of recurrence in less than 3% and of graft loss in less than 1%, according to the Review Organ Procurement & Transplantation Network (OPTN)[58]. Among the virological markers predictive of success of retransplantation, we can include a reduction of more than 2log10 copies of BKV DNA per mL in comparison to the peak value following the immunesuppression reduction, as well as the persistent viral clearance before the retransplantation [59]. Several retrospective studies have evaluated the outcome of retransplantation and concluded that it can be considered acceptable, although the risk of graft loss tends to be higher in comparison to a second kidney transplant in the absence of PVAN.

4. Polyomavirus-Associated Nephropathy in Native Kidney from Other Solid Organ Transplant Recipients

PVAN has been reported in native kidney of other solid organ transplant recipients, including liver, lung, heart, and pancreas. In these contexts, BKV-associated nephropathy should be considered in the differential diagnosis in the occurrence of recent onset of renal insufficiency. BKV viremia is not routinely monitored in other solid organ transplantation; when a diagnosis of PVAN is made, viremia usually ranges between 10^4 and 10^6 copies/mL. In these cases, a reduction of immunesuppression is indicated [60–68]. Few cases of BKV nephropathy have been also reported in native kidneys of AIDS-patients, closely related to deep immunesuppression [69].

5. Polyomavirus BK in Hematopoietic Stem Cell Transplantation

BKV reactivation in HSCT is quite common. Viruria and viremia have been reported during follow-up in up to 47%-94% and 23%-53% of HSCT-recipients, respectively. Reactivation may be associated to hemorrhagic cystitis (HC) with an incidence rate of 6% to 29%, particularly in the first year following transplantation, with approximately 15% of cases of HC being III/IV grade [70–73].

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Hematuria and/or interstitial nephritis may also occur. Hemorrhagic cystitis post-HSCT presents with dysuria, urgency, and lower abdominal pain in the presence of grade II-IV hematuria. Among other non-infectious and infectious causes of HC in HSCT, polyomavirus may be included, in addition to Adenovirus and Cytomegalovirus [74]. Considering virological markers of BKV replication in HSCT, viruria higher than 10^{10} copies/die (or 10^7 copies/mL) for approximately 30 days (range, 2-118) is considered predictive of onset of post-HSCT HC [75], whereas levels of viremia higher than 10^4 copies/mL for approximately 10 days are considered as related to a high risk of occurrence of post-engraftment HC [76]. Diagnosis of BKV-associated HC in this context is usually made on the basis of clinical presentation, exclusion of other causes and evaluation of markers of viral replication (viruria and/or viremia). Routine monitoring of BKV DNA (viruria or viremia) in HSCT is not performed.

Considering hematuria and/or interstitial nephritis in HSCT, in a study by O'Donnell and coll. on 124 HSCT recipients, a significant correlation between BKV viruria and hematuria was found, as well as a significant correlation between viremia and serum Creatinine increase. Two patients evidenced persistently positive viremia with development of nephropathy requiring hemodialysis [77].

6. Conclusions

PVAN still represents a potential treat in renal transplantation. Pathogenesis mainly results from immunosuppression with a combination of virus-induced graft damage and lack of adequate host immune responses. Diagnosis is usually made by histopathological evaluation, taking into account the potential for false negative results. Monitoring of viral replication is routinely recommended and may be useful from a presumptive diagnosis of kidney damage and the need for a preemptive modulation of immunosuppression. Knowledge of viral biology could be useful for an accurate evaluation of virological diagnosis and monitoring.

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Abbreviations

The following abbreviations are used in this manuscript:

PVAN	Polyomavirus-associated nephropathy
HSCT	Hematopoietic Stem Cell Transplantation
HC	Hemorrhagic Cystitis

References

1. Furmaga, J.; Kowalczyk, M.; Zapolski, T.; Furmaga, O.; Krakowski, L.; Rudzki, G.; Jaroszy, A.; Jakubczak, A. BK polyomavirus – biology, genomic variation, and diagnosis. *Viruses* **2021**, *13*, 1502.
2. Gardner, S.D.; Field A.M.; Coleman, D.V.; Hulme, B. New human papovavirus (B.K.) isolated from urine after renal transplantation. *Lancet* **1971**, *1*, 1253–1257
3. Padgett, B.L.; Walker, D.L.; ZuRhein, G.M.; Eckroade, R.J.; Dessel, B.H. Cultivation of papova-like virus from human brain with progressive multifocal leucoencephalopathy. *Lancet* **1971**, **1**, 1257–1260.
4. Allander, T.; Andreasson, K.; Gupta, S.; Bjerkner, A.; Bogdanovic, G.; Persson, M.A.; Dalianis, T.; Ramqvist, T.; Andersson, B. Identification of a third human polyomavirus. *J Virol* **2007**, *81*, 4130–4136.

 doi:10.20944/preprints202509.0099.v1

5. Gaynor, A.M.; Nissen, M.D.; Whiley, D.M.; Mackay, I.M.; Lambert, S.B.; Wu, G.; Brennan, D.C.; Storch, G.A.; Sloots, T.P.; Wang, D. Identification of a novel polyomavirus from patients with acute respiratory tract infections. *PLoS Pathog* **2007**, *3*, e64.
6. Feng, H.; Shuda, M.; Chang, Y.; Moore, P.S. Clonal integration of a polyomavirus in human Merkel cell carcinoma. *Science* **2008**, *319*, 1096–1100.
7. Schowalter, R.M.; Pastrana, D.V.; Pumphrey, K.A.; Moyer, A.L.; Buck, C.B. Merkel cell polyomavirus and two previously unknown polyomaviruses are chronically shed from human skin. *Cell Host Microbe* **2010**, *7*, 509–515.
8. van der Meijden, E.; Janssens, R.W.; Lauber, C.; Bouwes Bavinck, J.N.; Gorbalenya, A.E.; Feltkamp, M.C. Discovery of a new human polyomavirus associated with trichodysplasia spinulosa in an immunocompromized patient. *PLoS Pathog* **2010**, *6*, e1001024.
9. Scuda, N.; Hofmann, J.; Calvignac-Spencer, S.; Ruprecht, K.; Liman, P.; Kühn, J.; Hengel, H.; Ehlers, B. A novel human polyomavirus closely related to the african green monkey-derived lymphotropic polyomavirus. *J Virol* **2011**, *85*, 4586–4590.
10. Buck, C.B.; Phan, G.Q.; Raiji, M.T.; Murphy, P.M.; McDermott, D.H.; McBride, A.A. Complete genome sequence of a tenth human polyomavirus. *J Virol* **2012**, *86*, 10887.
11. Lim, E.S.; Reyes, A.; Antonio, M.; Saha, D.; Ikumapayi, U.N.; Adeyemi, M.; Stine, O.C.; Skelton, R.; Brennan, D.C.; Mkakosya, R.S.; et al. Discovery of STL polyomavirus, a polyomavirus of ancestral recombinant origin that encodes a unique T antigen by alternative splicing. *Virology* **2013**, *436*, 295–303.
12. Korup, S.; Rietscher, J.; Calvignac-Spencer, S.; Trusch, F.; Hofmann, J.; Moens, U.; Sauer, I.; Voigt, S.; Schmuck, R.; Ehlers, B. Identification of a novel human polyomavirus in organs of the gastrointestinal tract. *PLoS ONE* **2013**, *8*, e58021.
13. Mishra, N.; Pereira, M.; Rhodes, R.H.; An, P.; Pipas, J.M.; Jain, K.; Kapoor, A.; Briese, T.; Faust, P.L.; Lipkin, W.I. Identification of a novel polyomavirus in a pancreatic transplant recipient with retinal blindness and vasculitic myopathy. *J Infect Dis* **2014**, *210*, 1595–1599.
14. Gheit, T.; Dutta, S.; Oliver, J.; Robitaille, A.; Hampras, S.; Combes, J.D.; McKay-Chopin, S.; Le Calvez-Kelm, F.; Fenske, N.;
15. Cherpelis, B.; et al. Isolation and characterization of a novel putative human polyomavirus. *Virology* **2017**, *506*, 45–54.
16. Moens, U.; Calvignac-Spencer, S.; Lauber, C.; Ramqvist, T.; Feltkamp, M.C.W.; Daugherty, M.D.; Verschoor, E.J.; Ehlers, B. ICTV Virus Taxonomy Profile: Polyomaviridae. *J Gen Virol* **2017**, *98*, 1159–1160.
17. Kamminga, S.; van der Meijden, E.; Feltkamp, M.C.W.; Zaaijer, H.L. Seroprevalence of fourteen human polyomaviruses determined in blood donors. *PLoS ONE* **2018**, *13*, e0206273.
18. Mackenzie, E.F.; Poulding, J.M.; Harrison, P.R.; Amer, B. Human polyoma virus (HPV)--a significant pathogen in renal transplantation. *Proc Eur Dial Transplant Assoc* **1978**, *15*, 352-60.
19. Coleman, D.V.; Mackenzie, E.F.; Gardner, S.D.; Poulding, J.M.; Amer, B.; Russell, W.J. Human polyomavirus (BK) infection and ureteric stenosis in renal allograft recipients. *J Clin Pathol* **1978**, *31*, 338-47.
20. Harrison, P.; Mackenzie, E.F.; Poulding, J.M. BK virus in search of a disease. *Lancet* **1978**, *25*, 1150.
21. Gardner, S.D.; MacKenzie, E.F.; Smith, C.; Porter, A.A. Prospective study of the human polyomaviruses BK and JC and cytomegalovirus in renal transplant recipients. *J Clin Pathol* **1984**, *37*, 578-86.
22. Purighalla, R.; Shapiro, R.; McCauley, J.; Randhawa, P. BK virus infection in a kidney allograft diagnosed by needle biopsy. *Am J Kidney Dis* **1995**, *26*, 671-3.
23. Hirsch, H.H.; Randhawa, P.; AST Infectious Diseases Community of Practice. BK polyomavirus in solid organ transplantation. *Am J Transplant* **2013**, *13 Suppl 4*, 179-88.
24. Kotla, S.K.; Kadambi, P.V.; Hendricks, A.R.; Rojas, R. BK polyomavirus-pathogen, paradigm and puzzle. *Nephrol Dial Transplant* **2021**, *29*, 587-593.
25. Kazory, A.; Ducloux, D.; Chalopin, J.M.; Angonin, R.; Fontanière, B.; Moret, H. The first case of JC virus allograft nephropathy. *Transplantation* **2003**, *76*, 1653–1655.
26. Wen, M.C.; Wang, C.L.; Wang, M.; Wang, M.; Cheng, C.H.; Wu, M.I.; Chen, C.H.; Shu, K.H.; Changet, D; et al. Association of JC virus with tubulointerstitial nephritis in a renal allograft recipient. *J Med Virol* **2004,** *72*, 675–678.
27. Cavallo, R.; Costa, C.; Bergallo, M.; Messina, M.; Mazzucco, G.; Segoloni, G.P. A case of ureteral lesions in a renal transplant recipient with a co-infection of BK virus and JC virus. *Nephrol Dial Transplant* **2007**, *22*, 1275.

28. Sharma, N.; Abdulkhalek, S. Kidney Allograft Dysfunction Due to John Cunningham (JC) Virus Nephropathy. *Cureus* **2022** *14*, e32021.
29. Zhang, H.; Luo, J.Q.; Zhao, G.D.; Huang, Y.; Yang, S.C.; Chen, P.S.; Li, J.; Wu, C.L; Qiu, J.; Chen, X.T.; Huang, G. Concurrent JCPyV-DNAemia is correlated with poor graft outcome in kidney transplant recipients with Polyomavirus-associated Nephropathy. *Transplantation* **2024,** *108,* 1802-1811.
30. Gerber, J.S.; De Marchi, S.; Gaspert, A.; Fehr, T.; Cippà, P.E. JC Polyomavirus Nephropathy: A Rare Complication Late after Kidney Transplantation. *Case Rep Nephrol Dial* **2024**, *14*, 148-157.
31. Tomb, R.M.; McManus, S.K.; Kipgen, D.; Yaqub, S.; Taylor, S.; Gunson, R.N. JC Polyomavirus-Associated Nephropathy Case Report: Clinical and Laboratory Learning. *Br J Biomed Sci* **2025**, *82*, 14170.
32. Yang, D.; Keys, B.; Conti D.J.; Foulke, L.; Stellrecht, K.; Cook, L.; Lopez-Soler, R.I. JC polyomavirus nephropathy, a rare cause of transplant dysfunction: Case report and review of literature. *Transpl Infect Dis* **2017,** *19*
33. Sahragard I.; Yaghobi, R.; Mohammadi, A.; Afshari, A.; Pakfetrat, M.; Karimi, M.H.; Pourkarim, M.R. Impact of BK Polyomavirus NCCR variations in post kidney transplant outcomes. *Gene* **2024**, *913*, 148376.
34. Hirsch, H.H.; Randhawa, P.S.; on behalf of AST Infectious Diseases Community of Practice. BK polyomavirus in solid organ transplantation—Guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. *Clin Transplant* **2019**, *33*, e13528.
35. Kotton, C.N.; Kamar, N.; Wojciechowski, D.; Eder, M.; Hopfer, H.; Randhawa, P.; Sester. M.; Comoli, P.; Tedesco-Silva, H.; Knoll, G.; Brennan, D.C.; Trofe-Clark, J.; Pape, L.; Axelrod, D.; Kiberd, B.; Wong, G.; Hirsch, H.H.; on behalf of The Transplantation Society International BK Polyomavirus Consensus Group. The Second International Consensus Guidelines on the Management of BK Polyomavirus in Kidney Transplantation. *Transplantation* **2024**, *108*, 1834-1866.
36. Eder, M.; Kainz, A.; Omic, H.; Aigner, C.; Copic, D.; Kotton, C.N.; Kamar, N.; Wojciechowski, D.; Hirsch, H.H.; Oberbauer, R. A systematic literature review and meta-analysis evaluated modifiable risk factors for the development of BK polyoma virus-associated complications. *Kidney Int* **2025**, *S0085*-2538(25)00506-X.
37. Nast, C.C. Polyomavirusnephropathy: diagnosis, histologic features, and differentiation from acute rejection. *Clin Transplant Res* **2024**, *38*, 71-89.
38. Yamada, Y.; Tsuchiya, T.; Inagaki, I.; Seishima, M.; Deguchi, T. Prediction of early BK Virus infection in kidney transplant recipients by the number of cells with intranuclear inclusion bodies (decoy cells). *Transplant Direct* **2018**, *4,* e340.
39. Xing, J.; Procop, G.W.; Reynolds, J.P.; Chiesa-Vottero, A.; Zhang, Y. Diagnostic utility of urine cytology in early detection of polyomavirus in transplant patients. *J Am Soc Cytopathol* **2017**, *6*, 28-32.
40. Kant, S.; Dasgupta, A.; Bagnasco, S.; Brennan, D.C. BK virus nephropathy in kidney transplantation: a state-of-the-art review. *Viruses* **2022**, *14*, 1616.
41. Costa, C.; Bergallo, M.; Sidoti, F.; Astegiano, S.; Terlizzi, M.E.; Mazzucco, G.; Segoloni, G.P.; Cavallo, R. Polyomaviruses BK- and JC-DNA quantitation in kidney allograft biopsies. *J Clin Virol* **2009,** *1,* 20-3.
42. Randhawa, P.; Ho, A.; Shapiro, R.; Vats, A.; Swalsky, P.; Finkelstein, S.; Uhrmacher, J.; Weck, K. Correlates of quantitative

a. measurement of BK polyomavirus (BKV) DNA with clinical course of BKV infection in renal transplant patients. *J Clin Microbiol* **2004**, *42,* 1176-80.

43. Costa, C.; Bergallo, M.; Astegiano, S.; Terlizzi, M.E.; Sidoti, F.; Segoloni, G.P.; Cavallo, R. Monitoring of BK virus replication in the first year following renal transplantation. *Nephrol Dial Transplant* **2008,** *23*, 3333-6.
44. Huang, G.; Wang, C-X.; Zhang, L.; Fei, J-G.; Deng S-X.; Qiu, J; Li, J.; Chen, G-D.; Fu, Q.; Chen, L-Z. Monitoring of polyomavirus BK replication and impact of preemptive immunosuppression reduction in renal-transplant recipients in China: a 5-year single-center analysis. *Diagn Microbiol Infect Dis* **2015**, 81(1):21-6.
45. Costa, C.; Bergallo, M.; Sidoti, F.; Astegiano, S.; Terlizzi, M.E.; Mazzucco, G.; Segoloni, G.P.; Cavallo, R. Polyomaviruses BK- and JC-DNA quantitation in kidney allograft biopsies. J Clin Virol 2009, 44, 20-3.
46. Zanotto, E.; Allesina, A.; Barreca, A.; Sidoti, F.; Gallo, E.; Bottino, P.; Iannaccone, M.; Bianco, G.; Biancone, L.; Cavallo, R.; Costa, C. Renal Allograft Biopsies with Polyomavirus BK Nephropathy: Turin Transplant Center, 2015-19. Viruses 2020, 12, 1047.
47. Bateman, A.C.; Greninger, A.L.; Atienza, E.E.; Limaye, A.P.; Jerome, K.R.; Cook, L. Quantification of BK Virus Standards by Quantitative Real-Time PCR and Droplet Digital PCR Is Confounded by Multiple Virus Populations in the WHO BKV International Standard. *Clin Chem* **2017**, *63*, 761-769.

 doi:10.20944/preprints202509.0099.v1

48. Demey, B.; Aubry, A.; Descamps, V.; Morel, V.; Le, M.H.H.; Presne, C.; Brazier, F.; Helle, F.; Brochot, E. Molecular epidemiology and risk factors associated with BK and JC polyomavirus urinary shedding after kidney allograft. *J Med Virol* **2024**, *96*, e29742.
49. Zhang, H.; Luo, J-Q.; Zhao, G-D.; Huang, Y.; Yang, S-C.; Chen, P-S.; Li, J.; Wu, C-L.; Qiu, J.; Chen, X-T.; Huang, G. Concurrent JCPyV-DNAemia is correlated with poor graft outcome in kidney transplant recipients with Polyomavirus-associated Nephropathy. *Transplantation* **2024**, *108*, 1802-1811.
50. Grellier, J.; Hirsch, H.H.; Mengelle, C.; Esposito, L.; Hebral, A.L.; Bellière, J.; Weissbach, F.; Izopet, J.; Del Bello, A.; Kamar, N. Impact of donor BK polyomavirus replication on recipient infections in living donor transplantation. *Transpl Infect Dis* **2018,** *20*, e12917.
51. Hisadome, Y.; Noguchi, H.; Nakafusa, Y.; Sakihama, K.; Mei, T.; Kaku, K.; Okabe, Y.; Masutani, K.; Ohara, Y.; Ikeda, K.; Oda, Y.; Nakamura, M. Association of Pretransplant BK Polyomavirus antibody status with BK polyomavirus infection after kidney transplantation: a prospective cohort pilot study of 47 transplant recipients. *Transplant Proc* **2020,** *52*, 1762-1768.
52. Solis, M.; Velay, A.; Porcher, R.; Domingo-Calap, P.; Soulier, E.; Joly, M.; Meddeb, M.; Kack-Kack, W.; Moulin, B.; Bahram, S.; Stoll-Keller, F.; Barth, H.; Caillard, S.; Fafi-Kremer, S. Neutralizing antibody-mediated response and risk of BK virus-associated nephropathy. *J Am Soc Nephrol* **2018**, *29*, 326-334.
53. François, C.; Tinez, C.; Brochot, E.; Duverlie, G.; Castelain, S.; Helle, F.; Fiore, T.; Morel, V.; Touzé, A.; Sater, F.A.; Dakroub, F.; Akl, H.; Presne, C.; Choukroun, G.; Guillaume, N. Impact of pre-graft serology on risk of BKPyV infection post-renal transplantation. *Nephrol Dial Transplant* **2022**, *37*, 781-788.
54. Hillenbrand, C.A.; Akbari Bani, D.; Follonier, O.; Kaur, A.; Weissbach, F.H.; Wernli, M.; Wilhelm, M.; Leuzinger, K.; Binet, I.; Bochud, P.Y.; Golshayan, D.; Hirzel, C.; Manuel, O.; Mueller, N.J.; Schaub, S.; Schachtner, T.; Van Delden, C.; Hirsch, H.H.; Swiss Transplant Cohort Study. BK polyomavirus serotype-specific antibody responses in blood donors and kidney transplant recipients with and without new-onset BK polyomavirus-DNAemia: A Swiss Transplant Cohort Study. *Am J Transplant* **2025**, *25*, 985-1001.
55. Udomkarnjananun, S.; Kerr, S.J.; Francke, M.I.; Avihingsanon, Y.; van Besouw, N.M.; Baan, C.C.; Hesselink, D.A. A systematic review and meta-analysis of enzyme-linked immunosorbent spot (ELISPOT) assay for BK polyomavirus immune response monitoring after kidney transplantation. *J Clin Virol* **2021**,*140*, 104848.
56. Lepore, M.; Crespo, E.; Melilli, E.; Cruzado, J.M.; Torija, A.; Grinyó, J.M.; Bestard, O. Functional immune monitoring of BK Virus and donor-specific T-cell effector immune responses to guide treatment decision-making after kidney transplantation; an illustrative case report and literature review. *Transpl Infect Dis* **2021**, *23*, e13495.
57. Costa, C.; Mantovani, S.; Piceghello, A.; Di Nauta, A.; Sinesi, F.; Sidoti, F.; Messina, M.; Cavallo R. Evaluation of polyomavirus BK cellular immune response by an ELISpot assay and relation to viral replication in kidney transplant recipients. *New Microbiol* **2014**, *37*, 219-23.
58. Organ Procurement & Transplantation Network. Available online: https://optn.transplant.hrsa.gov/ (accessed on 30 July 2025).
59. Dharnidharka, V.R.; Cherikh, W.S.; Neff, R.; Cheng, Y.; Abbott, K.C. Retransplantation after BK virus nephropathy in prior kidney transplant: an OPTN database analysis. *Am J Transplant* **2010**, *10*, 1312-5.
60. Haririan, A.; Ramos, E.R.; Drachenberg, C.B.; Weir, M.R.; Klassen, D.K. Polyomavirus nephropathy in native kidneys of a solitary pancreas transplant recipient. *Transplantation* **2002**, *73*, 1350-3.
61. Muñoz, P.; Fogeda, M.; Bouza, E.; Verde, E.; Palomo, J.; Bañares, R.; BKV Study Group. Prevalence of BK virus replication among recipients of solid organ transplants. *Clin Infect Dis* **2005,** *41*, 1720-5.
62. Barton, T.D.; Blumberg, E.A.; Doyle, A.; Ahya, V.N.; Ferrenberg, J.M.; Brozena, S.C.; Limaye, A.P. A prospective cross-sectional study of BK virus infection in non-renal solid organ transplant recipients with chronic renal dysfunction. *Transpl Infect Dis* **2006**, *8*, 102-7.
63. Loeches, B.; Valerio, M.; Pérez, M.; Bañares,R.;J Ledesma, J.; Fogeda, M.; Salcedo, M.; Rincón, D.; Bouza, E.; Muñoz, P.; BKV Study Group of the Gregorio Marañón Hospital. BK virus in liver transplant recipients: a prospective study. *Transplant Proc* **2009**, *41*, 1033-7.
64. Sahney, S.; Yorgin, P.; Zuppan, C.; Cutler, D.; Kambham, N.; Chinnock, R. BK virus nephropathy in the native kidneys of a pediatric heart transplant recipient. *Pediatr Transplant* **2010,** *14*, E11-5.
65. Zeng, Y.; Magil, A.; Hussaini, T.; Yeung, C.K.; Erb, S.R.; Marquez-Alazagara, V.; Yoshida, E.M. First confirmed case of native polyomavirus BK nephropathy in a liver transplant recipient seven years post-transplant. *Ann Hepatol* **2015**, *14*, 137-40.

 doi:10.20944/preprints202509.0099.v1

66. Lai, C.; Bleasel, J.; McGrath, J.; Majumdar, A.; Kirwan, P.; Anderson, L.; Strasser, S.; Gracey, D. BK Nephropathy as a cause of renal dysfunction in an ABO-incompatible liver transplant patient. *Transplantation* **2020**, *104*, e83-e84.
67. Mallavarapu, R.K.; Sanoff, S.L.; Howell, D.N.; Roberts, J.K. BK virus nephropathy in non-renal solid organ transplant recipients: Are we looking hard enough? *Clin Transplant* **2021**, *35*, e14265.
68. Dube, G.K.; Batal, I.; Shah, L.; Robbins, H.; Arcasoy, S.M.; Husain, S.A. BK DNAemia and native kidney polyomavirus nephropathy following lung transplantation. *Am J Transplant* **2023**, *23*, 284-290.
69. Ebraimi, N.; Baghdadi, M.A.; Zuppan, C.W.; Rogstad, D.K.; Abdipour, A. AIDS-associated BK virus nephropathy in native kidneys: a case report and review of the literature. *J Investig Med High Impact Case Rep* **2024**, *12*, 23247096241232202.
70. Cesaro, S.; Dalianis, T.; Hanssen Rinaldo, C.; Koskenvuo, M.; Pegoraro, A.; Einsele, H.; Cordonnier, C.; Hirsch, H.H.; ECIL-6 Group. ECIL guidelines for the prevention, diagnosis and treatment of BK polyomavirus-associated haemorrhagic cystitis in haematopoietic stem cell transplant recipients. *J Antimicrob Chemother* **2018,** *73*, 12-21.
71. Zhou, X.; Zhang, S.; Fan, J.; Zhu, X.; Hu, S. Risk factors for BK virus-associated hemorrhagic cystitis after allogeneic hematopoietic stem cell transplantation: A systematic review and meta-analysis. *Clin Transplant* **2023**, *37*, e15121.
72. Mendoza, M.A.; Imlay, H. Polyomaviruses After Allogeneic Hematopoietic Stem Cell Transplantation. *Viruses* **2025**, *17*, 403.
73. Hu, J.; Li, S.; Yang, M.; Xu, L.; Zhang, X.; Zhao, H.; Dong, H.; Huang, Y.; Fan, J.; Li, L. Incidence, risk factors and the effect of polyomavirus infection in hematopoietic stem cell transplant recipients. *J Int Med Res* **2017**, *45*, 762-770.
74. Obeid, K.M. Infections with DNA viruses, Adenovirus, Polyomaviruses, and Parvovirus B19 in hematopoietic stem cell transplant recipients and patients with hematologic malignancies. *Infect Dis Clin North Am* **2019**, *33*, 501-521.
75. Leung, A.Y.; Suen, C.K.; Lie, A.K.; Liang, R.H.; Yuen, K.Y.; Kwong, Y.L. Quantification of polyoma BK viruria in hemorrhagic cystitis complicating bone marrow transplantation. *Blood* **2001**, *98*, 1971-8.
76. Erard, V.; Kim, H.W.; Corey, L.; Limaye, A.; Huang, M.L.; Myerson, D.; Davis, C.; Boeckh, M. BK DNA viral load in plasma: evidence for an association with hemorrhagic cystitis in allogeneic hematopoietic cell transplant recipients. *Blood* **2005,** *106*, 1130-2.
77. O'Donnell, P.H.; Swanson, K.; Josephson, M.A.; Artz, A.S.; Parsad, S.D.; Ramaprasad, C.; Pursell, K.; Rich, E.; Stock, W.; van Besien, K. BK virus infection is associated with hematuria and renal impairment in recipients of allogeneic hematopoetic stem cell transplants. *Biol Blood Marrow Transplant* **2009,** *15*, 1038-1048.e1.