Polyomavirus – an emergent pathogen in transplant recipients

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ABSTRACT
Medical centers that work with transplants often face opportunistic infections that demand specific tools to make diagnosis. The prevalence of latent polyomavirus infections is high, and the most common site of latency of the most prevalent polyomavirus in humans, BK virus (BKV), is the renal tissue. Hence, renal transplanted patients are particularly vulnerable to the damage caused by viral reactivation during immunosuppression. In such patients BKV is associated to ureteral stenosis and/or BKV nephropathy, leading to progressive dysfunction and graft loss, often diagnosed as rejection. In other organs recipients (namely lung, liver, heart and pancreas), BKN is also the most important clinical manifestation, whereas in bone marrow recipients the most common is hemorrhagic cystitis. This review presents the viral biology and discusses the pathophysiology of polyomavirus diseases and the diagnostic efficacy of the laboratory tests available, guiding to the best strategy for assessment and monitoring of patients at risk or under specific treatment.

Keywords: Polyomavirus; Polyomavirus infections/diagnosis; Virus diseases; Kidney diseases; BK Virus; Transplants

RESUMO
Centros que trabalham com transplantes freqüentemente deparam-se com infecções oportunistas cujo diagnóstico depende de ferramentas específicas. A prevalência de infecções latentes pelos vírus da família dos poliomavírus é alta e o sítio preferencial de latência do representante mais prevalente em humanos, o BK vírus (BKV), é o tecido renal. Desta forma, os pacientes submetidos a um transplante de rim são especialmente vulneráveis aos danos de uma reativação viral durante a imunossupressão. Nestes pacientes, o BKV tem sido associado a estenose ureteral e/ou a nefropatia associada ao BKV (BKN), que resulta em disfunção progressiva e perda do enxerto, frequentemente confundido com rejeição. Em receptores de pulmão, fígado, coração e pâncreas, também a BKN é a principal manifestação clínica, enquanto em receptores de medula óssea, o mais comum é cistite hemorrágica. A presente revisão apresenta a biologia viral e discute a fisiopatologia das doenças causadas por poliomavírus e a eficácia diagnóstica dos testes laboratoriais disponíveis, orientando para a eleição da melhor estratégia de investigação e monitorização dos pacientes de risco ou sob terapia específica.

Descritores: Polyomavirus, Infecções por polyomavirus/diagnóstico; Viroses; Nefropatias;Vírus BK; Transplantes

INTRODUCTION
In the age of transplants, management of immunodepressed patients brought with it the need to think of opportunistic infections, responsible for high morbidity and mortality rates of the transplanted population. Bacteria and fungi of low pathogenicity for the immunocompetent population and, most specially, virus that may become
latent, are agents that constantly put at risk patients with primary or acquired immunodeficiency.

The prevalence of latent infections due to the virus of the polyomavirus family is high in the general population. The renal tissue is the site of latency of its most important representatives with potential pathogenicity in humans - JCV and BKV. Renal transplanted patients are therefore particularly vulnerable to the damage caused by viral reactivation during prophylactic and therapeutic immunosupression against rejection. Nevertheless, any transplant recipient, as a consequence of immunosupression, is vulnerable to that risk.

Clinical suspicion and the possibility of a definite and early diagnosis of a polyomavirus infection are crucial to define treatment and prognosis. The knowledge of viral biology, of pathophysiology of the diseases caused by polyomavirus, of the factors that affect reactivation, and of diagnostic efficacy of several laboratory tests, enable defining the best strategy for the early diagnosis of BKV infection and monitoring of specific treatment(1).

This review presents the biological basis and viral behavior under the light of clinical setting with the objective of improving interpretation of laboratory results and helping choose the best investigation strategy and monitoring of patients at risk.

**Polyomavirus Family**

The polyomavirus family includes two potentially pathogenic virus for humans - JC virus (JCV) and BK virus (BKV)(2). There is a third virus, well known for its pathogenic potential in Rhesus monkeys, SV40, the first virus of the family to be identified as a contaminant of poliomyelitis vaccines(3).

All polyomaviruses, BKV, JCV and SV40, are serologically and genetically different, although having some genetic homology among themselves(2,4).

**GENOMIC STRUCTURE**

The virion of the polyomavirus family is small (40-45 nm in diameter), has an uncovered icosahedral capsid, 72 pentameric capsomers, a superhelical circular double-stranded DNA, with a molecular weight of 3.2x106 and 5Kb in size(5). The double strand of the viral genomic DNA represents approximately 12% of virion mass and has four cellular nucleosomal histones - H2A, H2B, H3 and H4, altogether called viral minichromosome(5).

BKV, JCV and SV40 are very homologous in the nucleotide sequences. JCV genome shares 75% of the sequence with BKV and 69% with SV40 genome sequence(2).

BKV genome consists of a coding region genetically preserved and a hypervariable noncoding regulatory region of 300 to 500 bp, known as NCRR(6).

**Coding Region**

The coding region is functionally divided in early and late regions, the former transcribed before and the latter after viral replication.

**Early Region**

The genes of the early region codify T (tAg) and t (tAg) antigens, transcribed before DNA replication and expressed soon after the host cell infection. TAg are essential activating factors for the viral DNA replication, regulating viral genome transcription and replication. Thus, TAg regulates its own transcription, and is responsible for cell transformation induced after BKV infection(6). TAg modulates the cell signaling pathway to induce the host cells into the S phase of the cell cycle, controlling cycle progression and apoptosis of the infected cells. The role played by tAg in the life cycle of the polyomavirus is not very clear(7).

**Late Region**

The late region genes codify: a) structural proteins of the capsid (VP1, VP2, and VP3) that unite to the replicated viral DNA to form virions(7) and b) agnoproteins that take part in the cell release of the replicated virus. The proteins VP1, VP2 and VP3 are mainly transcribed after the onset of genomic replication(6).

Since viral replication and virion reunion occur in the infected cell nucleus, proteins VP1, VP2 and VP3 are in the intranuclear compartment(4). VP1 is the largest capsid protein and accounts for more than 70% of virion protein mass. It mediates viral connection to the receptors in the susceptible cells and has neutralizing epitopes that inhibit hemagglutination; moreover, it has singular immunologic determinants as well as others, shared with the host cells. Proteins VP2 and VP3 are the smallest proteins of the capsid and have only structural function(5).

Agnoproteins differ from all other proteins codified by early and late regions for they re primarily located in the cytoplasm and perinuclear region of the infected cells. This concept of intracellular distribution suggests that agnoproteins participate in the viral capsid reunion, cell lysis and viral release of the host cell(4).

**Noncoding Regulatory Region (NCRR)**

The NCRR contains the replication origin (ori) and codifies in numerous regulatory factors involved in viral transcription and replication(6).

Figure 1 shows a scheme of the BKV genomic structure.
ASSOCIATION BETWEEN MUTATIONS AND GENOTYPES WITH DISEASE

BKV shows high antigenic variability, especially in the viral capsid protein VP 1 region, which allows the determination of different antigenic serotypes. Genetic instability apparently affects immune regulation of host, and may compromise both the pathogenesis of nephropathy caused by BKV and the development of antiviral agent resistance.

Viral transmission

The mode of viral transmission resulting in prime infection is not yet known. Although BKV will hardly be retrieved from respiratory tract, the fast acquisition of antibodies in early years in life suggests it could be transmission pathway.

Most recently, transplacental transmission has also been proposed. Other body fluids may also be potentially involved in the transmission of BKV infections, such as nasopharyngeal aspirates in children admitted to hospital due to severe respiratory infection, as well as semen and blood from healthy donors. BKV DNA can also be isolated from genital tract tissue and normal skin.

Based on the fact that viral genoma has frequently been detected in healthy livers, organ donors may also be considered an important mode of viral transmission before transplant.

Viral infection

The pathogenesis of polyomavirus infection can be described as follows: the virus enters host cells, viral genoma is multiplied, viremia follows and reaches out target organs, and multiplication takes place at those specific sites.

Viral capsid protein VP1 interacts with the specific receptors found in susceptible cells and mediates the uptake of virions through endocytosis. Glycosylated proteins – abundantly expressed in cellular surface – seem to act as receptors for VP1. Endosome releases virion in cytoplasm to then enter the nuclear compartment through a nuclear pore. Virus genoma replication takes place in that compartment to then spread by the hematogenous route leading to onset of infection at target organs.

EPIDEMIOLOGY

Primary infection

BKV and JCV are commonly found viruses. In the general adult population, over 80% show serologic evidence of previous exposure. Such exposure is essentially non-damaging, except when the host is immunologically depressed. Primary infections are typically asymptomatic, and persist indefinitely as latent infection in target organs.

Most commonly found clinical findings in primary infections are unspecific respiratory symptoms. The occurrence of tonsillitis suggests that the lymphoid tissue associated to oropharyngeal mucosa may be a site of primary infection. Neurological manifestations, such as Guillain-Barré syndrome and encephalities are rarely associated to BKV. Urogenital tract is BKV preferred latency site in humans.

Infection reactivation

Viral reactivation may occur at different points in time, especially under immunosuppression, when viral replication is high and the immune system is unable to refrain it. The most common clinical conditions for reactivation are transplants, primary or acquired immunodeficiencies, patients under immunotherapy for
malignant diseases, pregnancy, chronic disease patients (for instance, diabetes), and elderly patients.

The initial site of reactivation is the urinary tract and its earliest manifestation is asymptomatic virus urine excretion. Depending on reactivity level – which is to say, viral replication strength – the virus may be excreted in urine without having entered blood stream and be kept in urine, or develop into viremia and the onset of the disease.

BKV active infections are commonly associated to genitourinary tract conditions. JCV is not commonly found, and highly associated to the development of progressive multifocal leukoencephalopathy – a central nervous system fatal disorder described in AIDS patients.

**POLYOMAVIRUS INFECTION IN TRANSPLANTS**

**Bone marrow transplantation**

Viral reactivation is reported by 50% to 60% of bone marrow recipients. Prevalence is higher among allogeneic transplant recipients. The most commonly described clinical conditions described for polyomavirus infections among bone marrow transplant recipients are progressive multifocal leukoencephalopathy, hemorrhagic cystitis, hepatic dysfunction and pneumonitis.

Hemorrhagic cystitis is commonly reported in those patients. Early presentation on days immediately after transplant usually implies drug toxicity. Late onset hemorrhagic cystitis, however – between 2 and 10 weeks after transplant and persisting for a period longer than 7 days - is usually associated to BKV infection.

**Renal Transplant**

In the renal transplant scenario, the prevalence of latent polyomavirus infection is high. BKV infection has been reported to be as high as 65% among renal transplant recipients. BKV disease, in turn, usually affects approximately 10% of that population of patients.

Differently from what has been observed among bone marrow transplant recipients, manifestations associated to that type of infection after renal transplant are typically the development of interstitial nephritis, ureteral stenosis, systemic infection, or bladder cancer. The suspicion of polyomavirus disease should be raised when a renal transplant recipient presents macroscopic hematuria, urinary tract obstruction, or persistent increase in creatinine levels with no apparent cause. Ureteral stenosis is usually a late occurrence as a result of ureteral ulceration, and affects up to 5% of patients with viruria.

Most episodes of interstitial nephritis due to BKV – also called BKV nephropathy (BKN) – occur in the first three months after renal transplant, although the disease may occur some years after transplantation. In our days, BKN is certainly the most commonly found viral disease affecting renal graft parenchyma (8 to 20 times more frequent than cytomegalovirus) and may also be observed in native kidneys of recipients of other organs.

Risk factors for the development of BKN are not well understood. Environmental factors and/or nutritional status, gender and elderly age are still under discussion as possible contributors for the development of BKV disease. The high number of HLA incompatibilities – due to increased graft rejection risk – raises the need for more powerful immunosuppression, thus decreasing cell immunity efficiency in fighting viral infection. However, the number of previous rejection episodes – as well as organ cold ischemia time - have not been related to the development of BKV disease.

Among all risk factors for the development of BKN, immunosuppression level, especially under the use of drugs such as mycophenolate mofetil and tacrolimus, is one of the most important. Tacrolimus – a powerful calcineurin inhibitor used as prophylaxis and treatment of severely acute rejection episodes – has been strongly associated to the development of polyomavirus disease, with immunosuppression scheme reports reaching as high as 70% of BKN patients.

BKV-related nephropathy diagnosis may be complicated by the coexistence of acute rejection since overlapping histological findings may hinder any differentiation between the two processes. This is a complex clinical condition, since treating one will aggravate the clinical course of the other: strong immunosuppression from treatment of acute rejection episodes provides the ideal setting for viral replication.

BKN prognosis is poor and directly associated to early diagnosis. Patients diagnosed at an early stage of infection and properly managed presented good graft function outcome. However, patients with late diagnosis end up losing the transplanted organ. Prevalence of graft loss in BKN diagnosed patients ranges from 45% to 70%.

Patients with graft loss due to BKN can still be submitted to relatively safe retransplantation. The risk of relapse does not seem to increase when compared to the first transplant. However, success may depend on viral infection level at the time of retransplant.

**Other Transplants**

For recipients of other organs – such as heart, lung, liver, and pancreas – BKN is also the main clinical manifestation of an active BKV infection. For different
levels of chronic renal failure of undefined cause, viruria may be observed in up to 25% of cases. Viremia is less frequent, and reports describe up to 7% of heart recipients\textsuperscript{(27)}.

BKN may develop in those patients’ native kidneys exhibiting the same clinical, pathological, and virological characteristics of the BKN observed in renal grafts, which may lead patients to end-stage renal failure (ESRF). \textsuperscript{(27)}

**DIAGNOSIS**

Considering that the risk population is highly susceptible to irreversible clinical conditions as well as to high morbidity rate, high sensitivity diagnostic strategy is mandatory for early identification of viral infections that may potentially develop towards the disease. Diagnostic strategy should also be specific enough to minimize the risk of graft loss from inappropriate immunosuppression reduction for viral replication control. Test choices should also contemplate risk and cost-effectiveness concerns so as to focus diagnostic efficacy and economic feasibility at each center.

Definitive BKN diagnosis is determined based on renal biopsy, when antigen or viral DNA identification is performed, and histological patterns are also characterized to guide prognosis for each case (as shown in table 1)\textsuperscript{(28)}. A body of less invasive, complementary techniques is available to help making diagnosis. Currently, optical and electronic microscopy, serology, the detection of viral cytopathic effect, and methods that identify specific antigens or viral DNA act as contributing tools for diagnosis and monitoring of BKN. Immunohistochemistry techniques have been applied to renal graft biopsy specimens and urinary sediment\textsuperscript{(29)}, Viral isolation through molecular techniques has been employed in blood\textsuperscript{(30)}, urine\textsuperscript{(29-30)} and biopsy\textsuperscript{(8)}, and served as a specific, sensitive tool for the diagnosis of BKV infections.

The information obtained from each test must be interpreted in the clinical and pathological setting. What has been discussed so far will be arguable when facing the request and the interpretation of specific tests for patients at risk. Therefore, material peculiarities – such as the absence of cells in the urinary sediment of end-stage renal failure patients, for instance - may significantly compromise investigation in patients suspected of BKN. Likewise, the different PCR techniques (nested, RFLP, RT-PCR, real-time PCR), the selection of antigenic or molecular target (Tag, protein VP1, agnoproteins, etc), as well as the specimen elected for viral investigation (urine, blood, or renal biopsy) are key factors for the differentiation between active and latent infection.

As a rule, screening involves the assessment of viral cytopathic effect through urinary sediment using Papanicolaou staining technique. Once decoy cells are identified in the sediment (as shown in Figure 2), investigation is to be carried out for viral DNA or mRNA in urine and blood. Although specific viruria identification has been considered for BKV elsewhere, (through PCR or immunohistochemistry), graft biopsy is mandatory for histological analysis when viremia is present, with BKV investigation through immunohistochemistry or specific PCR in renal parenchyma. Once diagnosis is made and treatment started, the same techniques must be used for the monitoring of disease resolution. Negative tests are to be observed first in peripheral blood, then in urine, and finally in renal parenchyma.

![Figure 2. Decoy cell detected in urinary sediment of patient with renal dysfunction by Papanicolaou staining technique. Magnification 1000x](image)

There is no specific antiviral treatment available for polyomavirus disease at this point in time. The alternative for BKN control is immunosuppression reduction aiming at viral reactivation control. The final objective is to prevent viremia progression and disease. The obvious implication associated to that strategy is the subsequent increase of rejection risk. Should both pathologies coexist, the best approach is to treat rejection first, followed by immunosuppression reduction\textsuperscript{(16)}.

A number of drugs – retinoic acid derivatives, DNA-gyrase inhibitors, cytosine arabinoside, and...
cidofovir included – have succeeded in inhibiting polyomavirus DNA replication(1). Replacing higher risk immunosuppressants with antiproliferative activity sirolimus has been shown as the alternative for viral replication control.

**CONCLUSION**

Only through further understanding of BKV biology will it be possible to develop diagnostic and therapeutic strategies with improved potential for efficacy and effectiveness, as well as better cost-effectiveness for the management of risk patients.

Paying close attention to accurate information as provided by each of the tests (cytology, PCR or immunohistochemistry), considering different technique modalities and respective antigens/genomic regions as identified, as well as selecting the specimen for virus investigation (urine, blood, or renal parenchyma), all have diagnostic relevance and may be employed in monitoring specific therapeutic response. However, laboratory tests must be ranked following sensitivity, specificity and technical complexity in the light of the clinical suspicion for each case.

**REFERÊNCIAS**


Erratum


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