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Viral Infections after Kidney Transplantation: CMV and BK

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Abstract

Opportunistic infections commonly occur during the first 6 months after kidney transplantation, including cytomegalovirus (CMV) and polyomaviruses. Viral pathogens such as CMV and polyomaviruses, JC or BK virus (BKV), are able to replicate in the kidney and/or cause systemic disease, and symptomatic infection with these agents can be associated with significant morbidity and mortality in immunocompromised hosts. While BK virus usually replicates in kidney transplant causing BK virus nephropathy (BKN) with characteristic decoy cells in the urine, CMV infection more often leads to systemic infection involving the gastrointestinal tract (GIT), lungs, or liver and can only sporadically be detected in renal transplant. In both cases, the disease is most often due to reactivation of a latent virus. Prevention and early treatment of posttransplant infection are therefore crucial with kidney transplant recipients. Since BKV viruria and viremia can be seen without renal injury and viral nephropathy, a diagnosis of BKN must be confirmed by renal biopsy. To date, preemptive treatment is the best strategy for CMV infection, while no available standard therapy, except for reduction of immunosuppression, is available for BKV infection.

Keywords: CMV, BK, cytomegalovirus, polyomavirus, viral infections, kidney transplantation

1. Introduction

CMV and polyomavirus infection is common in the human population and mainly remains asymptomatic through the life of healthy individuals. However, in immunocompromised individuals, such as kidney transplant recipients (KTRs), it can be associated with various complications, including direct systemic effects of viral infection, bacterial or fungal superinfection, viral infection of the transplanted kidney, and acute and chronic rejection, which consequently diminish patient and graft survival. Current preventive strategies in KTRs include preemptive therapy with valganciclovir or intravenous ganciclovir and universal prophylaxis with antivirals after kidney transplantation and for 1–3 months after treatment with antilymphocyte antibodies. Strategies to control established virus infection include decreasing immunosuppression, adding antivirals, and a combination of both [1–3].

BK virus nephropathy is the most common manifestation of BKV reactivation after renal transplantation, leading to loss of renal grafts in approximately 43% of patients. BKV viruria and viremia can be seen without renal injury and viral nephropathy, so renal biopsy remains the gold standard for definite BKN diagnosis. Therapeutic strategies of BKN management are still very limited, so screening
protocols in order to detect early BK reactivation are important. BKN might be successfully managed with a reduction of baseline immunosuppression but is potentially harmful since it may be associated with increased risk of rejection [4–6].

2. CMV infection

CMV is a double-DNA virus of the herpesvirus family transmitted via saliva, body fluids, or tissue. There are various, species-specific strains of cytomegalovirus [7]. Seroprevalence ranges between 30 and 70% in Europe and North America. Following primary infection, CMV establishes latency in myeloid progenitor cells and can be transiently reactivated in a healthy host without causing disease, similar to polyomaviruses. However, CMV reactivates frequently and causes disease in KTRs in the setting of immunocompromised, typically in the first 2–3 months after transplantation [8, 9]. CMV viremia in the 1–6 months after transplantation is significantly more frequent in KTRs older than 65 years.

Reinfection (primary infection with a different human strain) can also occur [10]. CMV infection is the most common infectious disease following solid organ transplantation, including kidney [1, 3].

In addition to the direct effects of viral infection, CMV infection and disease have been associated with acute and chronic rejection and diminished patient and graft survival [2]. The transplanted kidney itself is only rarely affected by CMV reactivation.

The greatest recognized risk factor for CMV disease is a serological mismatch between the donor and the recipient (the recipient is CMV IgG seronegative and the donor is CMV IgG seropositive: D+/R−). Furthermore, CMV D+/R+ and CMV D−/R+ transplantations are of intermediate risk for the development of disease, and CMV D−/R− transplantation is considered as low risk (<5% incidence) [11, 12].

2.1 Definition

After the resolution of primary infection, CMV establishes latent infection. CMV can present in KTRs as either active CMV infection or CMV disease [9, 13].

Primary CMV infection: CMV infection in a person who was previously CMV seronegative (negative IgM and IgG CMV antibodies).

Latent CMV infection: after the resolution of acute (or primary) infection, CMV establishes latent infection. Patients who are CMV seropositive (IgG CMV antibodies) have latent infection. Secondary, symptomatic disease may present later, reflecting either reactivation of latent CMV or, less commonly, reinfection with a novel exogenous strain.

Active CMV infection is defined by CMV virus replication in plasma (viral load, viremia). CMV infection can be asymptomatic or symptomatic. The degree of immunosuppression in KTRs may determine progress to CMV disease.

CMV disease is defined as the presence of detectable CMV in a clinical specimen accompanied by other clinical manifestations. CMV disease may manifest as either CMV syndrome or tissue-invasive CMV disease [3].

2.2 Clinical features of CMV disease

2.2.1 CMV syndrome

For a determination of CMV syndrome, CMV in plasma (quantitative PCR CMV DNA (PCR)) and the presence of at least one of the following symptoms and signs
of disease are necessary: fever $\geq 38^\circ C$, general signs (malaise, myalgia, arthralgias), leukopenia ($\leq 3.5 \times 10^9/L$), atypical lymphocytosis ($\geq 5\%$), and thrombocytopenia ($\leq 100 \times 10^9/L$). In a case of suspected CMV nephritis in KTRs, kidney graft rejection should always be ruled out [3].

2.2.2 Tissue-invasive disease

In a case of tissue-invasive CMV disease, evidence of particular tissue/organ involvement (hepatitis, colitis, pancreatitis, pneumonitis, nephritis, cystitis, etc.) is based on clinical symptoms and signs associated with a particular organ, positive quantitative PCR CMV DNA in plasma, and, in particular, on the presence of CMV in a given organ or tissue (detected by methods of isolation, histopathology, immunohistochemistry, or hybridization in situ). CMV invasive disease can be most frequently detected in the intestine (40%) followed by the liver (20%), lungs (10%), kidneys (5%), and eyes/brain (1%) [8]. For CMV encephalitis, it is sufficient to prove the presence of CMV in the liquor (PCR) and for CMV pneumonitis in bronchoalveolar flushing (PCR).

In suspected CMV retinitis, ophthalmological examination is sufficient for the diagnosis. In patients with tissue-invasive disease (particularly in CMV infection of the central nervous system, chorioretinitis, and in CMV infection of the gut), CMV viremia may be absent, so some more invasive diagnostics (lumbar puncture, sigmoidoscopy/colonoscopy) must be proceeded in case of clinical suspicion [14].

2.3 Diagnosis

In KTRs who present with signs and symptoms suspicious for CMV disease, laboratory confirmation is required to establish the diagnosis. A biopsy with histopathologic examination of tissue is occasionally necessary to diagnose tissue-invasive CMV disease.

A diagnosis of CMV infection is most often confirmed with nucleic acid testing using polymerase chain reaction (PCR) for the detection of CMV DNA. PCR is primarily used to evaluate blood, cerebrospinal fluid, and ocular or vitreous fluid, although various clinical specimens can be subjected to this assay.

Among other tests to detect CMV, the demonstration of CMV p65 antigen in circulating polymorphonuclear leukocytes in the buffy coat has been used both to monitor response to therapy and as a guide to starting treatment in some centers. Traditional viral cultures are rarely used to diagnose CMV [15].

The most common serologic tests that detect CMV antibodies (IgM and IgG antibody to CMV) are based on enzyme-linked immunosorbent assay (ELISA). A positive test for CMV IgG indicates that a person was infected with CMV at some time during their life. The presence of CMV IgM cannot be used by itself to diagnose primary CMV infection because IgM can persist for months after primary infection and because IgM can be positive in reactivated CMV infections [16].

On occasion histopathological confirmation of CMV disease is necessary to prove CMV organ-specific dysfunction.

2.4 Histological features of tissue-invasive disease

Productive CMV infection in the tissue is characterized by a cytopathic viral effect in the biopsy specimen of parenchymal organs and the presence of CMV-positive cells by immunohistochemistry or by in situ hybridization with antibody directed against the immediate early antigen. Additionally, CMV virions may be detected by electron microscopy [17].
In daily practice, CMV reactivation is most frequently detected in GIT biopsies, including the colon and stomach (*Figure 1*). In contrast to polyomaviruses, CMV invasive disease is only sporadically detected in transplanted kidney [18, 19]. Histological features of CMV replication-related lesions in native kidneys are similar to those in renal transplants [20, 21].

### 2.4.1 CMV disease in kidneys

CMV nephritis is characterized by virally induced direct tissue injury and by biopsy-proven cytopathic changes. Cytopathic changes are typically focal and detected in tubular epithelial cells or endothelial cells (*Figure 2*).

Three patterns have been observed: pattern I with large intranuclear inclusions in tubular epithelial cells with interstitial nephritis, pattern II with central large eosinophilic intranuclear inclusions in endothelial cells, and rarely, CMV infection may occur as acute glomerulonephritis (pattern III) [18]. CMV infection may also affect podocytes.

In the predominant tubular involvement, tubular CMV infection is usually accompanied by variable interstitial inflammation. In addition, monocyte inclusions in the interstitial infiltrate may be observed. Occasionally, a dense nodular mononuclear and plasma cell infiltrate is present in the interstitium, sometimes reminiscent of granuloma. Focal necrosis and microabscesses are rarely observed. Prominent tubulitis reminiscent of T-cell-mediated rejection characteristic in BKN is absent.

The involvement of endothelial cells is characterized by a central large eosinophilic intranuclear inclusions surrounded by a circumferential halo resembling a typical owl’s eye. Glomerular and peritubular capillary endothelial cells may
be infected. In some nuclei, a smudgy-appearing intranuclear inclusion can be detected. In the cytoplasm of viral-infected cells, there are sometimes small basophilic cytoplasmic viral inclusions. When endothelial cells are predominantly CMV-infected cells, tubular epithelium tends to be spared. In such cases, interstitial inflammation is not prominent [18].

Immunofluorescence with a standard panel of antibodies is usually unremarkable, only rarely are scarce glomerular IgG deposits detected [20–22].

CMV nephritis may be associated with concurrent antibody- and T-cell-mediated rejection in 30% of cases [22]. In contrast to polyomavirus, CMV often replicates in endothelial and inflammatory cells. Distinction between infection-driven inflammation and rejection may be difficult.

Immunomodulation of the immune response might be the most important indirect effect of CMV infection on kidney graft, rather than direct CMV nephritis.
It is considered to promote rejection episodes by stimulating a T-cell-mediated response. Reinke reported that 85% of patients with late-acute renal allograft rejection with otherwise symptomless CMV infection responded to ganciclovir therapy, which emphasized the indirect role of CMV infection on graft function [23]. CMV infection does not activate classic complement pathway nor trigger the deposition of complement factor C4d along peritubular capillaries; in the case of positive C4d deposition, concurrent ABMR should be considered.

2.4.2 CMV disease in gastrointestinal tract

Cytomegalovirus infection of the gastrointestinal tract is the most common manifestation of tissue-invasive CMV disease and is a significant cause of morbidity and mortality in the solid organ transplantation recipients. Patients usually present with esophagitis, colitis, and hepatitis; however, infection can occur anywhere in the gastrointestinal tract [17, 24, 25].
Mucosal ulceration was the most common endoscopic finding present in 75% of cases (Figure 3). Other endoscopic features include mucosal edema, hyperemia, and nodularity. In a renal transplant patient, cytomegalovirus infection may rarely present as a localized disease, such as inflammatory polyps [26].

Two histologic patterns of GIT tissue injury have been described. In the first form, viral inclusions are typically found in the glandular epithelium with little associated tissue reaction (Figure 4). In the second form, CMV inclusions are found in swollen endothelial and stromal cells, especially in areas of ulceration. Typically, mucosal erosion, ulceration, hemorrhage, necrosis, perforation, and/or fistula formation can be detected. CMV colitis is characterized by uneven inflammation in the lamina propria, with active changes and ulcers with abundant purulent exudate (Figures 3 and 5) [24].

In contrast to other organs, CMV infection in the colon does not always produce the diagnostic large cells with viral inclusions with owl’s eye appearance. Rather, the infected cells can be smaller, up to twice as big as their normal counterparts, and have small basophilic inclusions, often with no characteristic clear halo. They have been called “atypical inclusions” [27].

Diagnosis is usually by histopathology with immunohistochemistry or viral culture of tissue specimens; molecular assays such as quantitative PCR also often have a role (Figures 4 and 5).

However, there is little consensus on the specificity of PCR [28–30]. Since CMV typically produces latent infection residing in leukocytes, concern has been raised that positive PCR might therefore not necessarily reflect active disease in the colon but only latent infection. The use of colon tissue alone was therefore not widely considered to provide definitive proof of CMV colitis [13]. Zidar et al. observed good correlation among the density of positive cells by immunohistochemistry, the
morphology, and the number of viral copies by qPCR in IBD patients. Both immunohistochemistry and qPCR can therefore be successfully used for diagnosing CMV reactivation, at least in CMV reactivation in patients with IBD. The optimal sites for endoscopic biopsies to obtain specimens with the highest values of CMV are the base and the edge of ulcers [28, 31].

2.5 Prevention of CMV disease

CMV can be prevented in two ways: by prophylaxis and by preemptive treatment. Both options are effective for preventing CMV disease [32–34].

2.5.1 CMV prophylaxis therapy

CMV prophylaxis is widely used in the transplantation setting and has been associated with reductions in CMV disease, mortality, and graft rejection. Prophylaxis refers to the administration of antiviral drugs to all patients (universal prophylaxis) or to a subgroup of patients at higher risk of viral replication (specific prophylaxis) for a predetermined period of time. In KTRs, prophylaxis therapy aims to prevent CMV infection and, consequently, CMV-associated disease. According to current guidelines, universal prophylaxis is recommended in patients with high risk (i.e., those who have D+/R− CMV IgG or who have received T-cell depletion for induction prior to transplantation). Antiviral drug treatment should begin immediately after transplantation or after the use of antilymphocyte antibodies. Patients with low to intermediate risk can undergo preemptive treatment instead of prophylaxis [35].

Until recently, the emphasis on prophylaxis with prophylactic agents focused on early disease occurring in high-risk patients, with the duration of prophylaxis typically no longer than 3 months. Although early-onset CMV infection was usually sufficiently controlled, the reported incidence of delayed-onset CMV infection following the completion of a 3-month course of preventive therapy was high, and, consequently, prophylactic therapy in most centers was extended to 6 months in the group of KTRs at most risk (D+/R−) [36, 37].

Several medications are available: acyclovir, valacyclovir, intravenous ganciclovir, oral ganciclovir, and valganciclovir. Ganciclovir takes precedence over acyclovir. In a clinical setting, the most commonly used medication for prophylaxis is oral valganciclovir with dose adjustment according to kidney function [38].

The prophylaxis should be initiated immediately after transplantation. The decision on the duration of prophylaxis depends on the CMV serostatus of the donor (D) and recipient (R), of the organ transplant, and the degree of immune deficiency in the transplant recipient.

2.5.1.1 Prophylaxis in D+/R− recipient

In D+/R−, prophylaxis should last for 3–6 months. According to recent research, many transplant centers are opting for a 6-month prophylaxis, which has been associated with a significant decrease in the incidence of late CMV disease, compared to 3-month prophylaxis. Valganciclovir at a dosage of 900 mg orally once daily with the dose adjusted for renal function is used in most centers for a period of 6 months following transplantation.

2.5.1.2 Prophylaxis in D+/R+ or D−/R+

In D+/R+ or D−/R+, prophylaxis should last for 3 months. Extension to 6 months is suggested for KTRs who have received antilymphocyte antibody induction.
Valganciclovir at 900 mg orally once daily for 3 months following transplantation, with the dose adjusted for renal function, is the standard prophylactic therapy in most centers.

2.5.1.3 Prophylaxis in D−/R−

There is little risk of CMV infection in these patients. Precautions for transfusion of blood and blood products of CMV-positive donors are required [35].

2.5.2 Additional considerations in the prevention of CMV in kidney transplant recipients

2.5.2.1 CMV matching

Theoretically, a method of minimizing the risk of CMV infection would be to avoid transplantation of a seropositive organ into a seronegative recipient. Historically, before the advent of antiviral prophylaxis, many units avoided transplanting CMV-positive solid organs into CMV-negative recipients. However, given the shortage of donor organs, such an approach is difficult to practice in these settings.

One area in which CMV matching remains relevant is in the elective use of blood products. Where it is known that both donor and recipient are seronegative for CMV, leukodepleted blood and blood products are available and should be used to minimize the risk of primary infection [39].

2.5.2.2 Passive immunoprophylaxis

Passive immunoprophylaxis has been explored in solid organ transplantation in a number of randomized trials, whereby hyperimmune globulin provided significant overall protection from severe disease, with a reduced rate of CMV disease to approximately half of that seen in the placebo groups. Intravenous treatment is generally less convenient for the patient and health-care provider and carries the theoretical risk of transmitting blood-borne viruses [39].

2.5.3 Preemptive therapy

With quantitative monitoring of CMV DNA in plasma (viral load, viremia) once a week (sometimes twice a week), CMV viremia can be detected before the occurrence of symptomatic infection. However, the exact cutoff point of plasma CMV concentration to initiate preemptive treatment (from a few hundred to several thousand copies of CMV DNA in 1 ml of plasma) is not known. The decision to initiate preemptive treatment is therefore individual and depends mainly on the degree and duration of immunosuppression [40].

The benefits of this type of strategy are that fewer patients are exposed to antivirals and for a shorter period of time (fewer side effects, fewer interactions with other medicines, lower costs).

Intravenous ganciclovir (5 mg/kg every 12 h or a dose adjusted to creatinine clearance) is used for preemptive treatment in a patient with a high viral load (>50,000 copies of CMV DNA in 1 ml of plasma), in severe renal impairment, and in pediatric patients; otherwise, valganciclovir (900 mg every 12 h, or a dose adjusted to creatinine clearance) is recommended. If there is no CMV disease, the CMV viremia is checked for the first time after 7–10 days of preemptive treatment, afterward being monitored every 7–10 days. It is recommended to continue with preemptive therapy until two negative results of quantitative plasma PCR CMV DNA tests performed in a space of 7 days [40].
<table>
<thead>
<tr>
<th>Drug</th>
<th>Dosage</th>
<th>Action</th>
<th>Formulation</th>
<th>Common side effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ganciclovir</td>
<td>5 mg/kg bw iv; adjusted to eGF</td>
<td>Competitive inhibition of DNA synthesis catalyzed by the viral DNA polymerase</td>
<td>Leukopenia, thrombocytopenia, diarrhea, long-term reproductive toxicity</td>
<td></td>
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<td></td>
<td>* Oral preparation has low bioavailability</td>
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<tr>
<td>Valganciclovir</td>
<td>900 mg bd p.o.; adjusted to eGF</td>
<td>Rapidly metabolized into active form (ganciclovir) in the intestinal wall and liver; same action as ganciclovir</td>
<td>Leukopenia, thrombocytopenia, anemia, gastrointestinal toxicity</td>
<td></td>
</tr>
<tr>
<td>Foscarnet</td>
<td>90 mg/kg bw bd iv or 60 mg/kg bw every 8 h; adjusted to eGF</td>
<td>Inhibits activity to the viral DNA polymerase by binding to the pyrophosphate binding site and blocking cleavage of pyrophosphate from the terminal nucleoside triphosphate added to the growing DNA chain</td>
<td>Nephrotoxicity, electrolyte disturbances, neurotoxicity</td>
<td></td>
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<tr>
<td>Drug</td>
<td>Dosage</td>
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<td>Formulation</td>
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<tr>
<td>Cidofovir</td>
<td>5 mg/kg i.v. once a week for 2 consecutive weeks. Following induction dose, 5 mg/kg i.v. once every 2 weeks administer with probenecid to decrease side effects to the kidney</td>
<td>After conversion to active form, cidofovir diphosphate competitively inhibits DNA polymerase</td>
<td>Nephrotoxicity, neutropenia, teratogenicity, carcinogenicity, nausea, vomiting</td>
<td></td>
</tr>
<tr>
<td>Maribavir</td>
<td>To be determined</td>
<td>Inhibits UL97 kinase and stops viral maturation and egress</td>
<td>Taste disturbance; inferior at preventing CMV disease</td>
<td></td>
</tr>
</tbody>
</table>

*i.v.*, intravenous; *p.o.*, peroral; *eGF*, estimated glomerular filtration.

Table 1.
Drugs used in therapy of CMV disease
2.5.3.1 Guiding of preemptive therapy by measurement of CMV-specific T lymphocytes

The activity and concentration of CMV-specific lymphocytes in the blood have a decisive role in controlling CMV infection, especially in situations of increased risk of CMV reactivation or primary infection, such as after therapeutic use of antilymphocyte antibodies. The count of CMV-specific T lymphocytes allows a decision on preemptive treatment in a period when the viral load is still not critically increased.

Among the available methods, the most reliable predictor of viremia and disease is measurement of the blood concentration of T lymphocytes, which, after in vitro stimulation with CMV peptides, increasingly produce cytokines such as interferon gamma and interleukin 2. CMV-specific lymphocytes CD4 and CD8 are analyzed by the flow cytometry method (one of the most commonly used is a whole blood interferon gamma release assay QuantiFERON-CMV test marketed by an Australian company, Cellestis Inc., which measures the production of interferon gamma after stimulating the patient’s lymphocytes with CMV peptides) [41].

2.6 Treatment of CMV disease

Treatment is always indicated in case of active CMV infection (CMV viral syndrome) or in the presence of tissue-invasive CMV disease [42].

Intravenous ganciclovir is a gold standard for the treatment of CMV disease. In mild to moderate cases of the disease, oral valganciclovir was found to be non-inferior to intravenous ganciclovir. However, due to limited evidence, severe disease should be treated with intravenous ganciclovir. Acyclovir and valacyclovir are not indicated for treatment. The use of foscarnet as a first-line therapy is limited by its toxicity (mainly nephrotoxicity) (Table 1).

Drug resistance should be suspected in patients with persistent viral replication and/or clinical progression after 2–3 weeks of treatment. Ganciclovir-resistant CMV infection has been observed in 1–2% of kidney transplant recipients and is a result of the widespread use of antiviral prophylaxis and preemptive therapy. Drug resistance typically develops in CMV D+/R− patients and is also associated with high viral load, prolonged antiviral therapy, high level of immunosuppression (i.e., use of antilymphocyte antibodies), and suboptimal serum drug concentrations. Genotypic tests reveal characteristic viral mutants (UL97) associated with resistance [43].

Drug-resistant or refractory CMV disease occasionally responds to an increased dose of ganciclovir. In cases of genotypic resistance of CMV to ganciclovir, it is necessary to introduce combined treatment with ganciclovir and foscarnet (half or standard doses) or treat with foscarnet only [44].

The treatment should be continuous until viral eradication is achieved in two assays after a minimum of 2 weeks of induction treatment. Initial treatment with intravenous ganciclovir can be later replaced with oral valganciclovir. During the course of treatment, renal function must be promptly monitored. In most cases (especially in high viremia, a moderate to severe clinical course, ganciclovir resistance), it is necessary to reduce immunosuppressive therapy (especially antimetabolites, i.e., azathioprine or mycophenolate). The same applies in cases of recurrent CMV infection/disease [35].

In the case of high-risk patients, some authors recommend secondary prophylaxis after completion of treatment, although no consensus has so far been achieved on this approach [35, 45].
3. BK polyomavirus infection and disease in humans

Polyomaviruses are non-enveloped, double-stranded ubiquitous DNA viruses living in birds and mammals as natural hosts. The name indicates their ability to produce tumors (Greek poly- many, multiple; -oma, tumors), particularly in rodents and experimental models [46]. Seroprevalence in humans ranges from 20 to 90%, depending on the viral strain and patient age. It generally remains asymptomatic in the renourinary tract of healthy individuals, although may undergo periods of self-limiting transient asymptomatic activation with viruria and viremia, without causing disease [46]. However, in immunocompromised individuals, such as renal transplant recipients, it can be associated with various patterns of tissue injury, of which BK virus nephropathy is the most common.

Among approximately 18 polyomavirus strains, BK virus, JC virus, and simian virus (SV-40) have been considered to be pathogenic in humans. Infections with SV-40 were detected following the administration of contaminated polio vaccines in the late 1950s, without known clinical manifestation in humans [46]. BK virus was isolated in 1971 from a patient with ureteral stenosis after kidney transplantation and was named after the initials of the infected patient. Similarly, JC virus was named after a patient with progressive multifocal leukoencephalopathy. Both strains are characterized by productive viral infection with tissue injury, showing specific tropism for the renourinary tract or central nervous system [46, 47].

Recent studies have indicated that BK virus may be involved in the tumorigenesis of bladder carcinoma in renal transplant recipients and salivary gland inflammation and sclerosis in HIV patients [48, 49]. Trichodysplasia spinulosa-associated polyomavirus and Merkel cell carcinoma polyomavirus, recently detected new strains, may be related to proliferative lesions and neoplasms without productive viral replication [50].

3.1 BK nephropathy

PVN is a major causative agent in nephropathy after renal transplantation, affecting 1–10% of patients [51]. In the past, when immunosuppressive therapy was based mainly on cyclosporine, only sporadic PVN cases were reported. Although modern immunosuppressive drugs introduced after 1990 have enabled less rejection and improved allograft survival, they have been responsible for the occurrence of previously uncommon side effects, including PVN and hemorrhagic cystitis [47].

Before screening protocols for PV reactivation in renal transplant recipients were routinely used, PVN was usually diagnosed late after transplantation, in an advanced histologic stage, with chronic renal changes leading to allograft loss within 1 year in 50–90% of cases [4, 50]. Potential misdiagnosis of concurrent rejection resulting in increased immunosuppression might contribute to accelerated allograft failure.

3.2 Features of BKN

PVN is typically caused by the BK strain and only rarely by simultaneous activation of BK and JC viruses. The specific viral activation mechanisms remain unknown [47]. The transplant microenvironment may promote viral reactivation, because only sporadic detection of PV in native kidney of patients with other organ transplants or in immunodeficient patients has been reported [52, 53]. PVN also commonly occurs in patients with posttransplantation complications, including
delayed graft function and acute rejection. Other risk factors are male gender, older recipient age, diabetes, prolonged ureteral stent placement, smoldering subclinical graft inflammation, and/or abnormalities of dendritic cell and NK cell/T-cell activation. Relative over-immunosuppression by modern immunosuppressive drugs, though, is considered the main risk factor [47, 51, 54].

Polyomavirus infection represents serological or virological evidence of virus exposure without distinguishing among replicating, latent, and transforming patterns. Manifest viral disease is, however, defined as histological evidence of polyomavirus-mediated organ pathology and is mainly limited to immunocompromised patients, such as transplant recipients [47, 55, 56].
Recognition of BKN is critical, since the proper therapy is reduction, rather than enhanced immunosuppression.

3.3 Diagnosis of BKN

In order to confirm intrarenal BKV replication, renal biopsy remains the gold standard for a definitive diagnosis of BKN [51]. A minimum of two cores including the medulla are recommended to make a correct diagnosis, since in the early stage, viral inclusions may be present only in the medulla [5, 51, 57]. However, characteristic viral inclusion and tubular injury might be focally observed in the biopsy specimens, so PVN can be missed due to sampling error (Figure 6).

3.3.1 Morphological characteristics of BKN

BKN is morphologically characterized by intrarenal viral replication, mainly in tubular epithelial cell nuclei (intranuclear inclusions), causing tubular injury, shedding of tubular epithelial cells, and cell lysis (Figures 7 and 8). On immunofluorescence, focal immune complex-type granular deposition of Ig along the tubular basement membrane is sometimes found, indicating BK infection (Figure 9), although the biologic and clinical significance of this finding needs further evaluation [5].

Viral replication in tubular epithelial cells can induce various nuclear changes: an amorphous ground-glass inclusion body (type 1), a central irregular inclusion body surrounded by a halo (type 2), finely granular nuclear alterations (type 3), and vesicular changes with coarsely clumped viral inclusions (type 4) (Figure 10).

Figure 7.
BK virus nephropathy. Virally induced tubular epithelial cell injury and lysis in cortex (A, HE, 200x) and medulla (B, HE, 200x). Intranuclear viral inclusion bodies are observed (arrow).
In rare cases, the ascending PV infection can affect the parietal epithelial cells of Bowman’s capsule, mainly detected by immunohistochemistry (Figure 6).

Diagnostic confirmation can easily be achieved by immunohistochemistry (Figure 6) or immunofluorescence, with antibodies directed against the polyomavirus T antigen, VP capsid proteins, or detection of intracellular virions of 40–50 nm in diameter by electron microscopy (Figure 11) [5, 57].

In early stages of PVN with focal and minimal tubular changes without tubular injury and characteristic intranuclear inclusions, a diagnosis can only be established by immunohistochemistry with antibody directed against SV-40 T antigen (Figure 12). Later in the course of the disease, many cases of PVN may show numerous infected cells and an inflammatory lymphocytic infiltrate with tubulitis mimicking acute T-cell-mediated rejection (Figure 13). Advanced disease, detected late after transplantation, often shows marked interstitial fibrosis/tubular atrophy, while interstitial inflammation and viral replication may be variable (Figure 14).

### 3.4 Differential diagnosis of BKN

PVN must be differentiated from other rare viral infections, including CMV, herpes simplex virus, and adenovirus. CMV disease in transplant recipients is more frequent than BKN and usually affects the intestine, liver, or lungs but only rarely manifests as CMV reactivation in renal graft. Since the histological features of BKN...
may overlap with other viral infections, specific immunohistochemical staining is a sensitive tool for differentiating among BK, CMV, adenovirus, or herpes simplex viral infection. The main histological features of common transplant kidney viral infection are shown in Table 2.
Figure 11.
Intranuclear viral inclusions in a tubular epithelial cell (A). Intranuclear virions measuring 40–50 nm in diameter (B, electron micrographs).

Figure 12.
BKN grade 1. Early phase with only focal tubular injury. (A, HE, 100x) and few SV-40 positive cells on immunohistochemistry (B, SV-40, 100x).
However, the most important differential diagnosis, particularly in PVN after reduction of immunosuppression, remains T-cell-mediated acute rejection [6]. Careful correlations with clinical data, such as the presence of donor-specific antibodies, recent immunosuppression reduction, DNA viral load in the serum, and presence of decoy cells in the urine, provide additional information in order to make a correct diagnosis. Glomeruli and vessels must be carefully examined in order to exclude glomerulitis and vasculitis, which would strongly suggest concomitant rejection. C4d positivity and diffuse peritubular capillaritis outside the area of extensive interstitial inflammation, together with positive donor-specific antibodies (DSA), are consistent with concomitant antibody-mediated rejection.

A diagnosis of PVN and concomitant T-cell-mediated rejection after immunosuppression reduction is challenging and needs careful correlation of biopsy findings with the dynamics of BK viremia. Focal interstitial inflammation in the context of stable graft function and recently cleared BK viremia should be interpreted as residual BKN, but the same histology findings detected beyond 3 months after BK clearance, accompanied by a rise in serum creatinine, might rather point toward acute rejection.

### 3.5 Course of BKN

The natural course of BKN remains to be elucidated. Some authors have reported that biopsies obtained after reduction of immunosuppression during decrease of the...
plasma viral load may show severe interstitial infiltrate and tubulitis reminiscent of T-cell-mediated acute rejection, but the outcome of renal grafts was good despite prolonged reduction of immunosuppression without corticosteroid administration [4, 6, 58, 59]. Such patients typically presented with a transient increase in serum creatinine, accompanied by a decrease in plasma viral load, which finally disappeared [59]. Moreover, serum creatinine returned to the baseline level after a few months. In subsequent biopsies, the virus was cleared from renal tissue, and inflammation resolved without the presence of marked interstitial fibrosis. These authors have suggested that such tubulointerstitial nephritis might be immune reconstitution-associated graft inflammation, enabling the resolution of PVN.

The challenging concepts of immune reconstitution injury and extensive inflammation in resolving BKN after reducing immunosuppression need further investigation [6].
3.6 Clinical presentation and management of BKN

Various studies have indicated that different extents of BKN in the transplant may predict the clinical presentation and outcome of the disease [58, 60, 61].

In order to provide optimal diagnostic and prognostic information of BKN, the Banff working group on BKN proposed three clinically significant disease grades based on the severity of polyomavirus replication and the degree of interstitial fibrosis [47, 62, 63]. BK virus replication was defined as the histologic viral load, estimated by the % of virally infected epithelial cells detected by immunohistochemistry. It ranged from scattered SV-40-positive cells in BKN grade 1 to numerous in grades 2 and 3 (Figures 12–14). In addition to SV-40-positive cells, grade 3 is characterized by interstitial fibrosis, which is responsible for irreversible tissue injury leading to graft failure [5, 47, 62].

Disease grade may reflect the time of the diagnosis: BKN grade 1 was generally diagnosed in the first 5 months after transplantation, usually presenting with normal renal function and associated with a favorable outcome in 85–90% of cases. In contrast, grade 2 BKN was detected 6–12 months posttransplantation, characterized by elevated serum creatinine or acute graft injury leading to graft failure in 25% of cases. Finally, grade 3 was usually detected more than 12 months after transplantation, also associated with worsening of kidney function and graft failure in 50% of cases (Table 3).

Since BKN has limited treatment options, the early detection of PVN has a major impact on the prognosis of the disease and therefore on allograft survival. Early diagnosis of PVN is difficult, because early BKN stage does not show any signs of systemic infection, proteinuria, or hematuria. Renal function may remain normal transiently, particularly when only the medulla is involved [5].

3.6.2 Screening of PVN

To date, reduction of baseline immunosuppression remains the only potentially effective therapeutic strategy of BKN, but it is associated with an increased risk of rejection. It is considered that preemptive reduction of immunosuppression prior to

### Table 2.
Histologic features of CMV and polyoma BK viral lesions in transplanted kidney.

<table>
<thead>
<tr>
<th></th>
<th>Polyomavirus</th>
<th>Cytomegalovirus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viral inclusions</td>
<td>Type 1: an amorphous ground-glass inclusion body</td>
<td>Smudgy/ground-glass nuclear inclusions surrounded by typical halo-owl eye</td>
</tr>
<tr>
<td></td>
<td>Type 2: a central irregular inclusion body surrounded by nuclear halo</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Type 3: finely granular nuclear alteration</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Type 4: finely granular nuclear alteration</td>
<td></td>
</tr>
<tr>
<td>Viral replication tubules</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Endothelial cells</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Inflammatory cells</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Acute tubular injury</td>
<td>Rarely</td>
<td>Rarely</td>
</tr>
<tr>
<td>Interstitial inflammation</td>
<td>Focal to diffuse</td>
<td>Focal</td>
</tr>
<tr>
<td>Tubulitis</td>
<td>Mild to severe</td>
<td>Mild</td>
</tr>
</tbody>
</table>
the development of overt nephropathy might be beneficial [6, 51, 59]. Since unrecognized BKN diagnosed late after transplantation causes chronic tissue injury and graft failure, the goal of screening protocols and classification schemes of BKN is to characterize early disease grades that respond to therapeutic intervention and may heal without progressing to chronic graft injury.

The first step of viral reactivation shown in almost all patients is characterized by the detection of characteristic polyomavirus inclusion-bearing cells in the urine—decoy cells (Figure 15). Initial viruria may be followed by detection of BK virus in plasma and onset of BKN after a 6–12-week window in some patients but only in a minority (Figure 16) [51].

Current guidelines recommend a urinary cytology test in order to detect urinary decoy cells initially and then a plasma test by PCR if urinary decoy cells are consistently present [51]. While PVN is most commonly diagnosed in the first year after transplantation, urine screening at least every 3 months during the first 2 years and after antirejection treatment seems appropriate to cover the majority of PVN cases [51]. The cytology urine test is characterized by a high negative predictive value to rule out a diagnosis of BKN and reduce costs. In addition, a window between viral reactivation and BKN enables urine samples to be screened in time.

However, several studies have shown that only a variable number of patients with urinary shedding of virus progressed to BKN. Notably, BK viruria and even viremia may represent transient asymptomatic BK activation or may originate from extrarenal sites, usually along the lower urinary tract. In patients without biopsy-proven BKN, preemptive long-lasting reduction of immunosuppression could be potentially harmful due to increased risk of acute rejection [64].

<table>
<thead>
<tr>
<th>PVN disease grade</th>
<th>Viral load</th>
<th>Interstitial fibrosis</th>
<th>Renal function</th>
<th>Time of diagnosis after TX (months)</th>
<th>Favorable outcome (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1</td>
<td>Scattered SV-40-positive cells</td>
<td>No</td>
<td>Normal</td>
<td>4–5</td>
<td>85–90</td>
</tr>
<tr>
<td>Grade 2</td>
<td>Numerous</td>
<td>Less than 25%</td>
<td>Increased serum creatinine, renal failure</td>
<td>6–12</td>
<td>75</td>
</tr>
<tr>
<td>Grade 3</td>
<td>Numerous</td>
<td>More than 25%</td>
<td>Increased serum creatinine, acute renal failure</td>
<td>12</td>
<td>50</td>
</tr>
</tbody>
</table>

Table 3. Characteristics of different BKN grades regarding viral load, chronic tissue injury-Interstitial fibrosis, renal function, time of diagnosis after transplantation, and outcome.

Figure 15. Decoy cells in urine screening test.
3.6.3 Biomarkers of BKN

A plasma test by PCR detecting BK copies is currently the accepted biomarker for clinical application, although the exact range of viral load that would predict BKN cannot be defined. The majority of patients with more than 10,000 copies per ml DNA in 1 ml of plasma show BKN on renal biopsy, but some patients with hardly detectable BK virus copy numbers may have manifest BKN. Several studies have indicated that PCR-based BK viremia correlates only moderately well with the presence of BKN and severity of the intrarenal disease, ranging between 25 and 75% (Figure 15) [6, 57].

Several biomarkers had been proposed in order to enable noninvasive diagnosis of definitive BKN without the risk of renal biopsy; these include heat shock protein 90alpha, CXCL9, neutrophil gelatinase-associated lipocalin, urinary exosomal biomarkers, urinary VP1, and urinary Haufen [65–67].

Polyomavirus-Haufen are tight cast-like three-dimensional viral aggregates, detected by negative staining electron microscopy of a voided urine sample. Since polyomavirus-Haufen admixed with uromodulin is formed in the tubular lumens, they might specifically predict intrarenal disease, comparable to renal biopsy [68]. Recent studies have indicated that the titer of polyomavirus-Haufen tightly correlates with the degree of intrarenal polyomavirus replication, providing additional information on the severity of PVN [64]. The urinary polyomavirus-Haufen test may emerge as a sensitive and specific biomarker for intrarenal viral disease, with positive and negative predictive value higher than 90%. The limitations of this investigation include the relatively high cost, time-consuming procedure, and limited availability of electron microscopy in transplant centers.

BK virus VP1 mRNA and urinary exosomal miRNA biomarkers have been described as potential surrogate markers for the diagnosis of PVN, with high sensitivity and specificity for BKN [66, 67]. Detection of additional urine biomarkers...
not only offers additional strategies for noninvasive PVN diagnosis but might also predict graft outcome.

3.6.4 Treatment of BKN

Management of PVN is still very limited. Reduction of the baseline immunosuppression, as the common therapeutic strategy, may be risky due to the possibility of acute rejection and may not be successful in all patients. Namely, some patients with BK viremia subsequently develop definitive BKN despite preemptive reduction of immunosuppression [4]. On the other hand, prolonged reduction of immunosuppression may be associated with clinical acute rejection rates of 8–14% [5, 69]. Renal biopsy, although considered to be an invasive procedure, may provide additional information in order to diagnose concomitant vascular rejection.

Data concerning the frequency of concurrent PVN and rejection vary. Some authors consider inflammation to be part of immune reconstitution injury, with a very low risk of concomitant rejection, whereas others have diagnosed concurrent acute rejection in 10–15% of cases at the time of initial PVN diagnosis [6, 47, 63]. Additional corticosteroid treatment in patients with PVN and severe tubulointerstitial inflammation at the time of PVN diagnosis also remains controversial. Some authors believe that corticosteroid treatment interferes with efficient BK clearance from the graft although, on the other hand, it might decrease interstitial inflammation and subsequent interstitial fibrosis [59].

Biopsy-proven diagnosis of concurrent BKN and rejection reveals the therapeutic dilemma concerning treatment strategy. In some individual cases, concomitant biopsy-proven T-cell-mediated rejection and PVN on low immunosuppression have been efficiently treated with transient pulse immunosuppressive therapy [70]. On surveillance kidney biopsy, BK was cleared from the tissue, interstitial inflammation disappeared, and serum creatinine returned to the baseline level.

Many of the therapeutic agents, including leflunomide, quinolone, and cidofovir, have been involved in BKN treatment with undetermined antipolyomavirus effect. It was recently shown that intravenous immunoglobulins’ (IV IGs) administration may be effective in the treatment of BK viremia and PVN in patients who have failed to respond to immunosuppression reduction and leflunomide therapy [71].

Successful resolution of BKN and BK clearance may be associated with the recipient’s antiviral cell-mediated immune response. Recently, novel laboratory-based methods based on BK-directed cellular immunity and anti-BK T-cell phenotype have been introduced, such as ELISPOT assays, which might provide additional information in relation to the resolution of PVN [72–74].

4. Conclusions

KTRs receiving immunosuppressive regimes to prevent transplant rejection are at increased risk of opportunistic infections such as CMV and polyoma BK virus. In both viruses, reactivation of latent infection is the principal mechanism rather than de novo infection.

While reactivation of CMV infection is usually present with systemic infection, including fever, leukopenia, organ dysfunction, and viremia without invading renal graft, the most harmful presentation of BK infection reactivation includes BKN directly affecting the transplanted kidney.

Both CMV and BK infections commonly appear in the first year after transplantation, so screening protocols are very important in order to detect patients with
increased risk of virus reactivation and early disease, and this should be started immediately after transplantation.

With systematically quantitative monitoring of CMV DNA in plasma, CMV viremia can be detected before the occurrence of symptomatic infection. Ganciclovir and valganciclovir are generally used to prevent or treat CMV.

For BKN screening, current guidelines recommend a urinary cytology test initially and then plasma DNA test by PCR if urinary decoy cells are consistently found.

The reduction of baseline immunosuppression is considered to be the common therapeutic strategy of BKN but is associated with increased risk of rejection. Since polyomavirus viruria and viremia can be observed without renal injury and BKN, a definite diagnosis of PVN must be confirmed by renal biopsy. In order to prevent BKN in viremic patients, preemptive reduction of immunosuppression prior to the development of overt nephropathy might be beneficial.

Careful detection and management of opportunistic infection enable better graft survival and quality of life in KTRs.

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Conflict of interest

Authors declare no conflict of interest.

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