BK Virus Infection after Kidney Transplantation

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Polyomaviruses were said to be in search of a disease. Now it is well established that the BK strain of polyomavirus causes a nephropathy in kidney transplant recipients immunosuppressed with highly potent drugs such as tacrolimus and/or mycophenolate mofetil. The morphologic hallmarks of BK virus nephropathy are nuclear inclusion bodies in tubular epithelial cells and acute tubular injury, which cause allograft dysfunction. Risk factors of disease and clues to diagnose BK virus nephropathy and concurrent rejection are discussed in this review. BK virus nephropathy is generally preceded by the excretion of decoy cells (polyomavirus-infected cells) in the urine and accompanied by viremia. These findings serve as adjunct clinical markers of BK virus nephropathy which are incorporated into a proposed diagnostic and therapeutic algorithm for patient management.

Introduction

Polyomaviruses (BK and JC virus strains having human relevance) are without clinical significance in the immune-competent host. Primary infections with polyomaviruses are common. They occur early during childhood with only minor clinical symptoms and are independent for JC and BK viruses. Approximately 60% to 80% of adults in the Western world have serological evidence of a previous infection with polyomaviruses. JC virus and BK virus are tropic for epithelial cells in the kidney and, in particular, for the transitional cell layer of the urogenital tract. Here, they commonly remain latent without causing any illness. Along the transitional cell layer, latent JC virus can be found in approximately 88% and latent BK virus in 33% of individuals.[1] In 0.5% to 20% of immune-competent hosts, polyomaviruses can be periodically reactivated and reenter into a replicative cycle.[2-4] Such reactivation is transient, asymptomatic, and morphologically characterized by the detection of intranuclear viral inclusion bearing cells in the urine (coined *decoy cells*)[5] along with cell lysis (i.e., release of viral particles). Decoy cells appear to originate from the transitional cell layer. Such asymptomatic reactivation of polyomaviruses can be seen during pregnancy or in patients suffering from diabetes mellitus or malignant tumors. Renal function remains unaltered. These general aspects of polyomavirus infections in immune-competent individuals form the background against which pathophysiological aspects of disease continue to be studied.

Disease caused by polyomaviruses is seen only in immune-compromised hosts. Depending on the viral strain and the type of the underlying suppression of the immune system, different illnesses prevail. JC virus typically affects the brain and can cause progressive multifocal leukoencephalopathy (PML) in AIDS patients. Disease caused by BK virus is generally seen in the urogenital tract, including the kidneys (i.e., BK virus nephropathy [BKN]).[6-8] In the following review, we focus on BK virus nephropathy affecting renal allografts.

In the setting of kidney transplantation, one should bear in mind that the prevalence of latent infections with polyomaviruses is high. Thus, latent polyomaviruses are likely often passed in the graft from the donor to the host (who also may have latent virus in the GI tract). This constellation has not resulted in any clinically recognized complications.[9] Asymptomatic reactivation of polyomavirus-

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es (i.e., decoy cells in the urine) is also commonly seen in healthy kidney transplant recipients (up to 22% of patients, which is similar to findings made in immune-competent hosts). Such activation normally lasts for only a short duration (up to several weeks), and renal allograft function remains unaltered. Thus, latent polyomavirus infections lacking morphological and immunohistochemical signs of an infection have to be distinguished from transient, clinically asymptomatic reactivation of latent polyomavirus infections, which are morphologically characterized by decoy cells, and symptomatic viral disease (BKN). BKN is typically accompanied by signs of viral activation (decoy cells in the urine)—but not vice versa.[6,10,11]

Latency and activation are common phenomena, but disease is a rare event. This is why we avoid the generic term *infection*. The potential risk factors promoting viral disease are discussed below.

BKN shows viral replication in tubular epithelial cells. The last phase of the replicative cycle includes cell lysis of the host tubular cell, resulting in the release of viral particles into tubular lumens. Histologically, virally induced acute tubular injury and necrosis are seen (see below). It is likely that viruses spread primarily by receptor-mediated mechanisms from cell to cell via an ascending route starting in collecting ducts (i.e., spread to cell types for which the BK virus is tropic, such as tubular cells and parietal epithelial cells). This mode of replication of the BK virus has clinical significance in that it differs from other viruses (such as EBV). Whether the coactivation of BK virus and JC virus (up to 37% of cases with BKN) is clinically important remains to be determined.[12] Thus far, there is no solid evidence that BKN is caused by specific mutations in the BK virus strain.[13-16] Infections with the SV40 virus strain do not play any role.[8]

Prior to the mid-1990s, BKN did not gain considerable clinical attention and had been exceptionally rare in the 1970s and 1980s. At the University of Basel, we diagnosed our first case of BKN in October 1996. We confirmed that the diagnosis of BKN had not been missed in previous years by large-scale rescreening of archival biopsies from renal allograft recipients who had formerly been treated with high-dose cyclosporine or antilymphocytic preparations. Currently, BKN is by far the most common viral disease affecting renal allografts (approximately 10 to 20 times more frequent than CMV.[11] Thus, we are dealing with a new complication, and new risk profiles promoting BKN have to be considered.[6,7,10,11,17-23] The following review is primarily based on our experience with 29 renal allograft recipients (n = 89 biopsies) presenting with BKN.

BK Virus Nephropathy (BKN)

Synonyms

Synonyms for BKN include the following: polyomavirus infection, type BK; polyomavirus disease; polyomavirus-associated interstitial nephritis; and BK interstitial nephritis.

Clinical Data

In our center, BKN was diagnosed in the renal allografts 380 days (mean; median = 267 days, range = 40–1377 days) after transplantation. The patient population (n = 29) showed a slight male predominance that did not reach statistical significance: 9 women (mean age = 47, median = 52, range = 10–70 years) and 19 men (mean age = 49, median = 52, range = 27–70 years) (in 1 patient, the sex is unknown). Our observations are similar to those reported by others.[7,22-25] The current prevalence of BKN in different transplant centers varies between less than 1% and up to 5.5% in Basel.[22-24] In our experience and that of others, many patients presenting with BKN had a complicated posttransplantation course. Prior to the diagnosis of BKN, we examined a total of 33 biopsies from 15 of 29 patients. Fourteen patients (48.3% of the whole study population) showed biopsy-proven acute rejection episodes. Transplant endarteritis was significantly more often diagnosed (transplant endarteritis: 12/33 biopsies [36.3%] in comparison to controls: 9/70 biopsies [12.9%], difference p = 0.009, see Table 1; also see refs. 7,23,25–28). Frequently, high-dose immunosuppressive regimens containing tacrolimus and/or mycophenolate mofetil were being administered.[6,10,11,23,25,28] The observed differences in prevalence seem to be linked to the preferential (center-dependent) use of these newer immunosuppressive regimens. Centers preferring (high-dose) immunosuppression with tacrolimus

Table 1 | **Prevalence of Biopsy-Proven Acute Rejection Episodes before the Diagnosis of BKN**

Type of Rejection	Disease Group	Control Group	*p*-Value
Tubulo-interstitial			
Number	16/33	31/70	*ns*
%	48.5	44.3	
Transplant endarteritis			
Number	12/33	9/70	0.009
%	36.4	12.9	

Disease group: 33 biopsies from 15 patients who ultimately developed BKN. All 33 biopsies prior to the initial diagnosis of BKN were free of viral nephropathy. Control group: 70 time-matched control biopsies (median: 27 days posttransplantation) from 61 patients who never developed BKN.

and/or mycophenolate mofetil encounter high numbers of BKN cases,[7] in contrast to those still relying on conventional cyclosporine- and azathioprine-based protocols. Race and type of organ donation do not appear to carry any significant risk for promoting viral disease.[24] At the time of the initial diagnosis of BKN, patients clinically present with varying degrees of allograft dysfunction, which can be insidious at times (serum creatinine in our patients at time of diagnosis: mean = 267 µmol/l, median = 205, range = 133–516). Decoy cells are typically detected in the urine, and BK virus DNA can be found in the plasma by polymerase chain reaction (PCR) (see below).[6,10,11,16,20,23,25,26,29,30] These latter findings constitute a clinical risk profile, which can help with patient management (see Fig. 1). Glomerular proteinuria, significant hematuria, or signs of a generalized infection (i.e., fever) are typically absent.[7,11] BKN is normally not associated with hemorrhagic cystitis (with only one reported exception).[23] There is no convincing evidence that BK virus causes ureteral stenosis. In renal allograft recipients, BK virus generally does not spread to organs outside the graft (with one exception discussed elsewhere in this issue).

BKN has a major impact on graft survival, which is significantly poorer than in control patients free of viral nephropathy.[24] We and others had reported a rapid graft failure rate in up to 45% of cases (within 6 months after diagnosis; range = 1–17 months).[11,22] More recently, our results have improved, with no graft losses due to persistent (= ongoing) BKN in the past 2 years (in Basel: overall graft failure rate due to BKN is 28%, compared with a graft failure rate of 16.4% a year after the diagnosis of BKN at the University of Maryland Hospital).[24] Even if persistent (= ongoing) BKN does not lead to rapid graft loss, functional deterioration is always seen (in our experience, serum creatinine levels are, on average, 50% above baseline readings after 12 months of persistent disease).[11] However, there is also increasing evidence that patients can fully overcome BKN with complete cessation of viral replication (for more detailed information, also see below). Viral clearance was observed in 8 of 29 (27.5%) histologically confirmed cases in Basel, with clinical information available in 7 of 8 cases. Patients had overcome BKN 334 days after the initial diagnosis (mean; median = 115 days, range = 57–1003, based on repeat biopsies). Renal function was preserved in 5 of 7 patients (serum creatinine: mean = 185 µmol/l; median = 179, range = 126–260). Two of 7 patients with delayed viral clearance (> 1 year) lost graft function (1 patient on hemodialysis, the other one with a serum creatinine of 500 µmol/l).

Diagnosis and Morphology

The diagnosis of BKN must be established histologically by renal biopsy.[6,7,11,20] Other techniques (such as urine cytology or PCR studies) only serve as adjunct tools for patient management (see below). The morphological hallmarks of BKN are intranuclear viral inclusion bodies, seen exclusively in epithelial cells, and focal necrosis of tubular cells (Fig. 2). Due to the viral replicative mode, intranuclear inclusion bodies can always be found in cases of BKN, although they are sometimes scarce (see below). Four different variants of intranuclear inclusion bodies are seen, frequently side by side in the same tubule (Fig. 3):

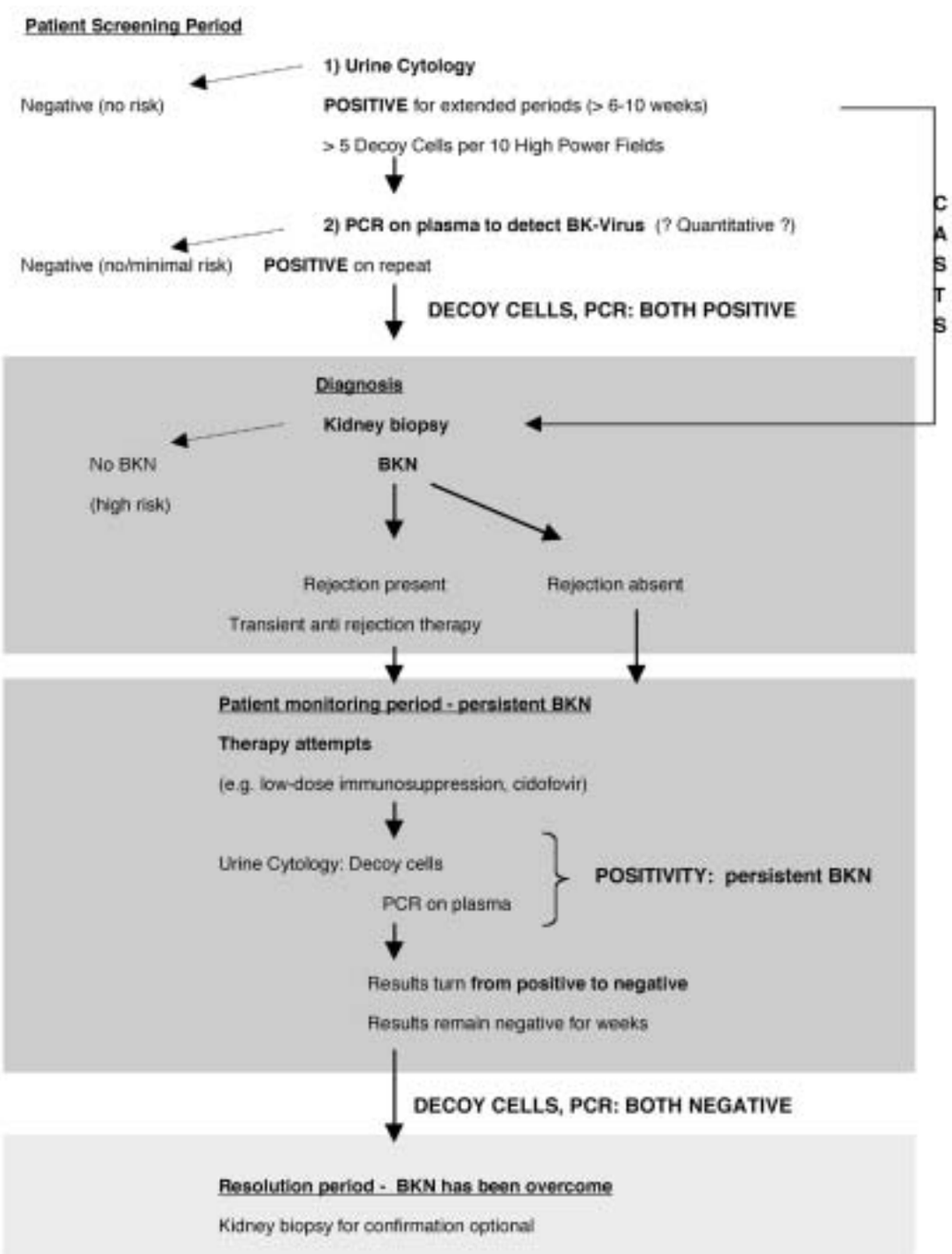

Figure 1. Diagnostic algorithm. Adapted from reference 26.

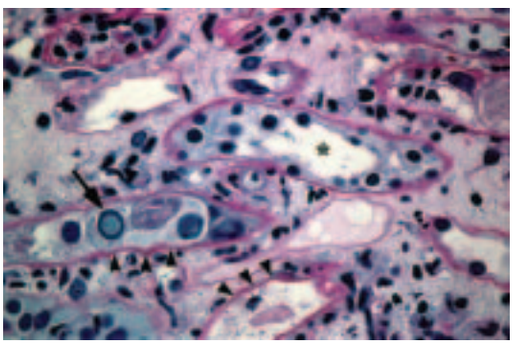

Figure 2. BKN (time of initial diagnosis = 18 weeks posttransplantation). A typical picture of virally induced focal acute tubular necrosis. Intranuclear viral inclusion bodies are seen in some tubular epithelial cells (arrow) associated with cell necrosis and denudation of the underlying tubular basement membranes (arrowheads). Note that despite severe tubular epithelial cell damage, the basement membranes remain intact (arrowheads). Adjacent distal tubules (asterisks) are unremarkable. PAS-stained section, 150× original magnification.

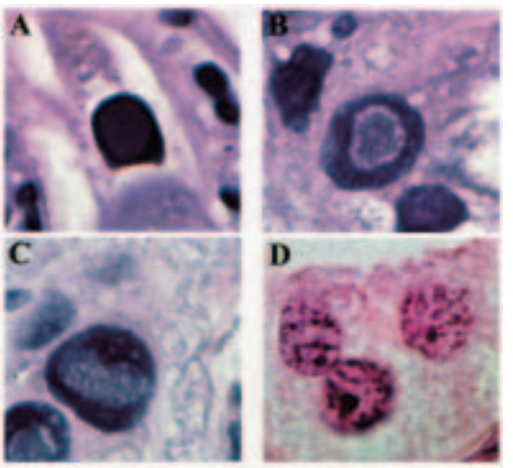

Figure 3. Different intranuclear viral inclusion bodies. (A) Type 1 inclusion body—an amorphous basophilic ground-glass variant. (B) Type 2—an eosinophilic, granular variant surrounded by a (mostly incomplete) halo. (C) Type 3—a finely granular variant without a halo. (D) Type 4—a vesicular variant with clumped, irregular chromatin. Hybrid variants of inclusion bodies exist. H&E-stained sections, 400× original magnification.

- Type 1 (the most frequent form)—an amorphous basophilic ground-glass inclusion body
- Type 2—an eosinophilic, granular type surrounded by a (mostly incomplete) halo
- Type 3—a finely granular form without a halo
- Type 4—a vesicular variant with markedly enlarged nuclei; clumped, irregular chromatin; and occasional nucleoli

Type 2 inclusions are morphologically similar to cytomegalovirus (CMV) inclusions. Type 4 inclusion-bearing cells resemble malignant tumor cells. "Hybrid variants" of different types of inclusion bodies are often seen. These different phenotypes likely represent various stages of viral replication and disintegration of nuclei and chromatin in association with fixation artifacts. Cells with cytopathic changes are often enlarged. Inclusion-bearing cells can be abundant in the medulla and distal tubules (possibly reflecting the ascending route of viral spread from the urothelium to the renal cortex).[6,31] As mentioned above, viral replication results in the release of viral particles by cell lysis. Thus, severe tubular epithelial cell injury and necrosis with denudation of basement membranes is a typical finding in BKN.[6,11] Despite marked epithelial cell damage, tubular basement membranes remain intact (Fig. 2). They can serve as a structural base for subsequent tubular regeneration. These morphological changes are not unique but rather represent acute tubular necrosis (ATN). ATN is the morphological correlate for allograft dysfunction. Viral inclusions can also be seen in parietal epithelial cells, with occasional small crescent formations.[6] In the renal pelvis and ureters, viral inclusion bodies (mostly type 1) can be detected in superficial transitional cells but rarely in the proliferating basal cell layer.[6] If inclusion-bearing transitional cells are sloughed into the urine, then they can easily be detected in cytological preparations as decoy cells (Fig. 4). Podocytes, endothelial cells, mesenchymal cells, and inflammatory cells are not infected by the BK virus in human allografts[6,11,20] (1 patient with massive involvement of endothelial cells, discussed elsewhere in this issue, is a unique exception).

Although the morphology is typical for BKN, it is not pathognomonic because other viruses such as herpes simplex virus, adenovirus, and (less likely) even CMV must be ruled out. Certain morphological clues can favor one diagnosis over another (such as frank tubular destruction, hemorrhage, and granulomatous inflammation in cases of aden-

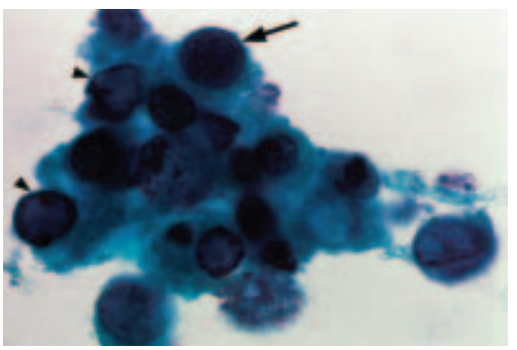

Figure 4. Decoy cells in urine cytology preparations. Inclusion-bearing epithelial cells (type 1 arrowheads and type 3 arrow) are easily recognized. Papanicolaou-stained section, 312× original magnification.

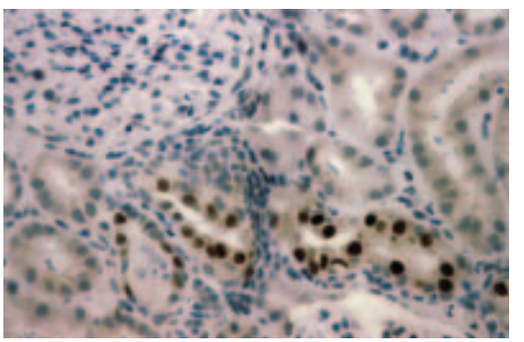

Figure 5. BKN—immunohistochemical detection of BK virus/polyomaviruses. A strong (brown) staining reaction is seen in the nuclei of some tubular epithelial cells. Formalin-fixed and paraffin-embedded tissue section, detection of SV40 T antigen (common to all known polyomaviruses). 70× original magnification.

ovirus infections or intracytoplasmic viral inclusions in cases of CMV). Diagnostic confirmation can easily be achieved by immunohistochemistry or electron microscopy. Ultrastructurally, polyomaviruses present as viral particles measuring between 30 and 50 nanometers in diameter, occasionally forming crystalloid structures. Immunohistochemistry can be performed on formalin-fixed and paraffin-embedded tissue sections using commercially available antibodies detecting the SV40 large T antigen, which is common to JC, BK, and SV40 viruses (Fig. 5). If required, BK virus-specific antibodies are available as well (for details with regard to the staining procedures, see the addendum). Also, in situ hybridization techniques may be used to detect amplified viral particles.[7] Of note: immunohistochemistry and electron microscopy only help with confirming the light microscopical impression. The routine use of immunohistochemistry on all renal allograft biopsies generally does not unveil any formerly undiagnosed cases of BKN.

Key histological features of BKN are tubular injury and necrosis (ATN; Fig. 2). ATN explains many aspects of the disease. If viral replication is limited, then tubular injury is very focal, and consequently renal function will not be severely altered. On the other hand, widespread viral replication involves many tubules, with a diffuse pattern of ATN resulting in severely impaired allograft function. If BKN is diagnosed early during the course of the disease and viral replication ceases quickly, then renal function may fully recover.[11,26] The background for this observation is the capacity of ATN to heal once injury stops. Mitotically active tubular cells can easily replace necrotic ones along intact segments of tubular basement membranes. Thus, restitution of the normal tubulo-interstitial architecture can be achieved. However, if BKN is diagnosed late (i.e., persistent viral replication over an extended period of time), then longstanding ATN results in irreversible interstitial fibrosis and tubular atrophy (late phenomena; Fig. 6).[6] Disease progression in BKN was carefully studied by Drachenberg et al.[31] In repeat biopsies, the authors could clearly document the natural progression of persistent viral nephropathy ranging from initial (mild) tubular injury (pattern A) to ultimate (severe) interstitial fibrosis and tubular atrophy resulting in graft failure (patterns B and C). Because BKN is often seen in patients with previous rejection episodes, viral changes may, of course, be superimposed on rejection-induced damage.

BKN, Interstitial Nephritis, and Rejection

The correct diagnostic interpretation of "inflammatory changes" in the setting of BKN is difficult and has led to considerable controversy.[6,7,11] Often, BKN is simply referred to as *BK virus interstitial nephritis*. Although this descriptive term accurately describes the findings, it implies that the inflammatory changes observed are secondary to the viral replication. Using this terminology, one major differential diagnosis of "inflammation" in a renal al-

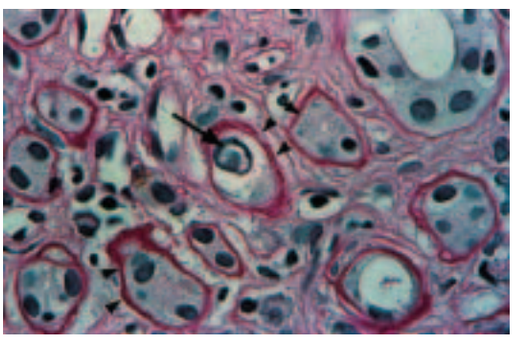

Figure 6. BKN (repeat biopsy taken during persistent BKN 56 days after the initial diagnosis). Focal interstitial fibrosis and tubular atrophy with thickened basement membranes (arrowheads) is seen. These changes are likely secondary to virally induced tubular injury (a viral inclusion body is still seen in the center, arrow). Tubular atrophy is irreversible. Graft function in this case was lost within 14 months after the initial diagnosis of BKN. PAS-stained section, 251× original magnification.

lograft—rejection—remains without further consideration. In our opinion, it is crucial to establish a diagnosis of rejection in the setting of BKN because patients are generally treated by lowering immunosuppression. A prerequisite for such an approach is the absence of rejection. This is why we preferentially use the term *BK virus nephropathy* to clearly distinguish viral disease from potential concurrent rejection. We have provided previous evidence that two diagnoses—rejection and BKN—can be rendered concomitantly.[11,26] We showed that initial, transient antirejection therapy, subsequently followed by low-dose immunosuppression, could result in complete viral clearance and full functional recovery.[26] Transient antirejection therapy (such as bolus steroids) has, in our experience, not resulted in "explosive" viral spread and replication. We think this two-step approach can clinically be very useful, but it is not yet widely used by others.[7]

How do we then address the diagnostic challenge of BKN and "interstitial inflammation"? In general, the inflammatory cell infiltrate seen in cases of BKN can vary considerably.[6,11] Sometimes, the inflammation is very scant or even absent, particularly in cases of "mild viral disease"—BKN pattern A (which is seen in 35% of biopsies at time of the initial diagnosis).[31] Occasionally, scattered polymorphonuclear leukocytes and mononuclear cell elements are encountered in and adjacent to severely altered tubules.[11] These changes are easily interpreted as "secondary" in nature. Whether plasma cell–rich interstitial infiltrates, which are sometimes seen,[6] represent a "specific" inflammatory response is currently undetermined. Different are cases with abundant mononuclear cells (lymphocytes and macrophages) in areas of the cortex lacking cytopathic changes, accompanied by marked tubulitis (particularly in tubules without viral inclusion bodies seen by light microscopy and immunohistochemistry; Fig. 7) or even transplant endarteritis. Most striking in these cases is the discrepancy between widespread lymphocytic infiltrates compared with only focal virally induced cytopathic changes (Fig. 7). In our experience, such cases represent BKN and concurrent rejection.[11,26] Further diagnostic confirmation can be achieved by employing well-established immunohistochemical markers of acute rejection:

1. Most important, the expression of major histocompatibility (MHC) class II molecules in tubular epithelial cells can help.
2. Occasionally, the detection of the complement degradation product C4d along peritubular capillaries can be useful diagnostically.

It has been previously reported that the expression of both markers remains unaltered by BK virus replication in tubular cells.[11,32] At the time of initial diagnosis of BKN, we found transplant endarteritis in 3 of 29 cases (10.3%), tubular MHC class II expression in 5 of 11 cases (45.5%), and capillary C4d deposits in 3 of 11 (27.3%) biopsies studied. These findings clearly demonstrate that rejection can coexist with BKN (also see Drachenberg et al.[31]). Therefore, attempts to diagnose rejection and concurrent BKN should be made.[26]

Adjunct diagnostic tools. Although the diagnosis of BKN has to be established in a graft biopsy, adjunct diagnostic tools are available to manage patients, including urinalysis (particularly the screening for decoy cells) and plasma PCR studies to search for BK virus DNA.

Decoy cells in urine cytology preparations. The search for decoy cells in urine is, in our experience and that of others, a very useful clinical tool that is

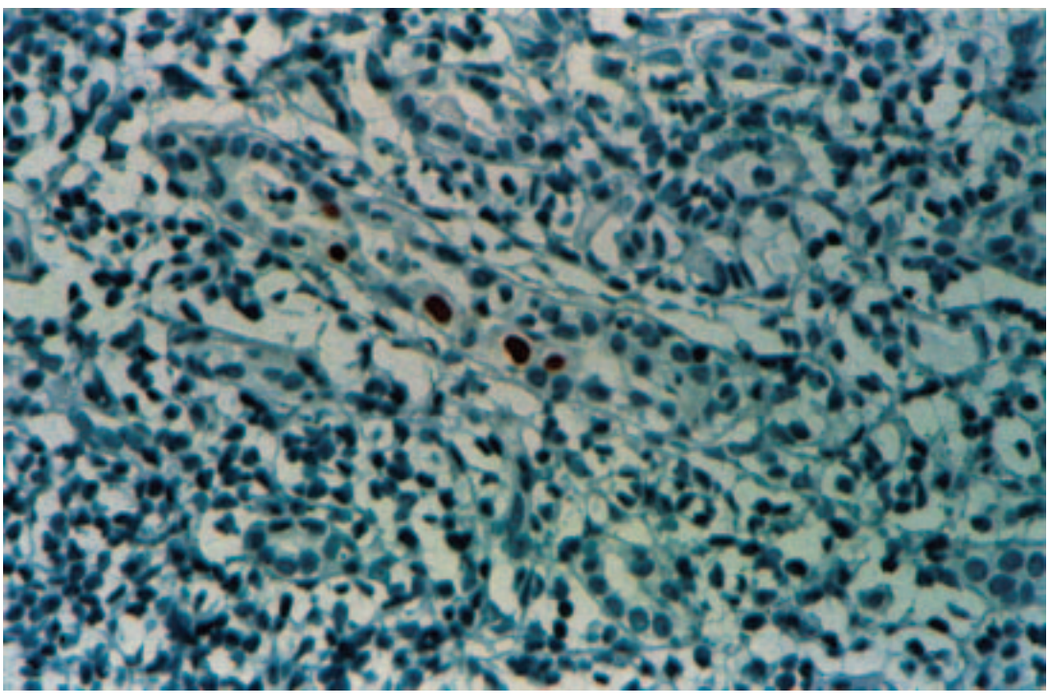

Figure 7. BKN and concurrent rejection (time of initial diagnosis, 28 weeks posttransplantation). The biopsy shows only very focal viral inclusions (brown color, highlighted by immunohistochemistry). In contrast, the interstitial compartment is diffusely infiltrated by mononuclear cell elements with tubulitis. Tubules expressed major histocompatibility (MHC) class II molecules, typically seen in cases of rejection. The complement degradation product C4d is not detected. Two diagnoses—BKN and tubulo-interstitial rejection—were rendered. The patient received initial antirejection therapy with bolus steroids followed by reduction of immunosuppression. A subsequent repeat graft biopsy after 6 months showed persistent viral disease. Graft function is still good. Immunohistochemistry to detect SV40 T antigen, 100× original magnification.

currently underappreciated (Fig. 4).[6,10,11,20,25] Decoy cell screening is an inexpensive and widely available analytic tool that can be performed by any pathologist worldwide without requiring special equipment. With some experience, decoy cells can also be detected by nephrologists in urine sediments.[10] Why are decoy cells helpful? As mentioned above, decoy cells (which typically contain BK virus) are a sign of activation of polyomaviruses in the urogenital tract. Activation of BK virus is, of course, a prerequisite for viral disease (BKN). All cases of BKN (including the current series of patients) had decoy cells in the urine at the time of initial diagnosis.[11,24,25] Once BKN had been overcome and viral activation had ceased, decoy cells disappeared from the urine.[11,31] In our laboratories, we set an arbitrary threshold of more than 5 decoy cells per 10 high-power fields (in cytology smears) for a urine specimen to qualify as "decoy cell positive." Using this approach, we established an overall sensitivity of decoy cells to predict BKN of 100%, a specificity of 95%, a positive predictive value of 27%, and a negative predictive value of 100% (in a retrospective analysis of more than 500 urine samples).[11] Thus, if patients are "decoy cell negative," they do not suffer from BKN (and other diagnoses should be considered). If, however, the urine is decoy cell positive, BK virus has been activated and the patient is at potential risk for developing BKN. This patient should be closely followed (such as with plasma PCR studies; see below). The predictive value of decoy cell–positive urines can be enhanced by incorporating additional parameters into the decision-making process: persistent detection of decoy cells in the urine (for more than 6–10 weeks), inflammation in the cytology smear background,[20] or

"positivity" in the setting of impaired allograft function.[31] Of note: the detection of decoy cell casts is diagnostic for BKN. We found no statistically significant association between the numbers of decoy cells shed and viral disease (in contrast to another report).[31] Based on this experience, we conclude that costly, highly sensitive urine PCR analyses requiring specialized laboratories do not contribute any significant information in addition to the "fast and cheap" decoy cell screening by urine cytology.

Plasma PCR Analyses to Detect BK Virus DNA

BKN is characterized by viral replication in tubular cells, epithelial cell injury, lysis, and ATN. BK virus likely gains access to the bloodstream through injured tubular walls and via peritubular capillaries. Indeed, PCR analyses to detect BK virus in plasma are useful clinical tools because positive PCR results mirror the time course of viral nephropathy. The patients we studied converted from plasma negative to plasma positive by PCR after transplantation, remained plasma positive during the course of histologically proven BKN, and then became plasma negative after BKN had been overcome.[30] In our experience and that of others, all patients with BKN had detectable BK virus in the plasma[23,29,30] in contrast to control groups.[29,30] However, the detection of BK virus in plasma can also yield false-positive results in renal allograft recipients without concurrent histological proof of BKN. Thus, plasma PCR studies to predict BKN have a sensitivity of 100%, a specificity of 88%, a positive predictive value of 82%, and a negative predictive value of 100%.[30] In combination with the screening for decoy cells in the urine, plasma PCR studies[33,34] are a valuable adjunct clinical tool for patient management. Using these adjunct tools, one should bear in mind that during the manifestation of BKN, decoy cell and PCR analyses generally become positive before the histological diagnosis of BKN may be established ("negative histological window";[6,11,30] see below). A recent study from our group (Hirsch et al.),[35] showed that patients with biopsy-proven BKN had more than 7700 copies of BK virus DNA per milliliter of plasma compared to patients without BKN, in whom the viral copy numbers remained low, in general below 2000 per milliliter.

Risk Factors

It is likely that BKN is promoted by a currently poorly defined multifactorial process. Because BKN is a new complication that typically affects the graft many months after transplantation, formerly unknown risk factors occurring during the posttransplantation period must be searched. What has changed in the world of kidney transplantation from the late 1980s until now? Most important, new potent immunosuppressive drugs—foremost, tacrolimus and mycophenolate mofetil—have been introduced into the immunosuppressive protocols. Their worldwide use has been linked to the outbreak of BKN.[10,22] Indeed, judged by the shedding of decoy cells, tacrolimus is more effectively activating latent BK virus than cyclosporine (6.5% of patients in comparison to only 2.5%, respectively; $p < 0.01$[1]). However, these findings alone are insufficient to explain the entire risk profile because only a small minority of renal allograft recipients treated with tacrolimus and/or mycophenolate mofetil ultimately developed viral nephropathy. Potentially, an additional factor facilitating viral replication is tubular cell injury itself. Tubular cell damage and differentiation have been shown to promote the replication of polyomaviruses in an animal model.[36] Thus, differentiating tubular cells such as those recovering from rejection episodes or ischemic-type damage in an allograft recipient treated with new highly potent immunosuppressive drugs may provide the right window for viral replication. Because many patients who ultimately developed BKN had suffered from preceding rejection episodes in our clinical experience (biopsy proven in 48.2%), this hypothesis seems to be an attractive possibility. Others have not made the same observation,[24] and certainly, many more risk constellations involving immunosuppression with tacrolimus and mycophenolate mofetil are conceivable—all of them are speculative and have not been carefully studied. For example, do these drugs increase the risk of BKN in a seronegative allograft recipient (without a latent infection) receiving a donor organ from a seropositive individual (graft with a latent infection)?[9] Alternatively, do mutations render the BK virus more virulent? Consequently, are mutant viral strains responsible for BKN?[9] The latter question is currently under investigation. So far, however, no

unique mutation common to all cases of BKN has been detected. On the basis of our current knowledge, we believe that risk factors promoting the reactivation and replication of polyomaviruses are key players in the pathogenesis of BKN.

Clinical Management

Virally induced ATN is the fundamental histological concept behind the pathophysiology of BKN. The degree of allograft dysfunction (if rejection is absent) is proportionate to the number of injured tubules (i.e., the degree of ATN; Fig. 2). Rejection, of course can have an additional impact on allograft function.[26] Virally induced ATN (BKN) may heal, and grafts can function well in the long run—with restitution of the normal architecture.[26] The duration of ATN determines the extent of irreversible secondary damage (i.e., interstitial fibrosis and tubular atrophy; Fig. 6). Interstitial fibrosis and tubular atrophy have a major impact on long-term graft function and survival.

The management of patients suffering from BKN is a very difficult clinical challenge, and despite all efforts, outcome is often ominous. At present, specific antiviral drugs to treat BKN are not available. Only very limited therapeutic attempts have been made with the nephrotoxic agent cidofovir[33] (for more detailed information, see other articles in this issue). Currently, the most frequently used clinical approach to overcome viral nephropathy is to lower baseline immunosuppression with the hope that the host immune system will subsequently "clear the virus." Prerequisites for lowering immunosuppression are as follows:

1. the absence of rejection at the time of the initial diagnosis of BKN, and
2. no viral stimulation of rejection during persistent nephropathy.

Because BKN and concurrent rejection can be diagnosed, the first objective can be met (see above). The second objective has been validated.[11,24]

In general, the clinical management of renal allograft recipients who are at risk for developing BKN has two major goals:

1. The diagnosis of BKN should be established early (when histological changes/ATN are potentially fully reversible and before interstitial fibrosis and tubular atrophy have occurred). This represents the *patient screening period*.
2. BKN should be "treated" long enough (presumably for many weeks) to achieve complete cessation of viral replication (i.e., full viral clearance). This represents the *patient monitoring period*.

Both objectives can be met with the help of surrogate markers (Fig. 1).[6,11,26,30] High-risk patients (e.g., those coming out of rejection episodes and being treated with tacrolimus and/or mycophenolate mofetil) should be regularly screened. The screening period should include the search for decoy cells in urine cytology preparations and PCR analyses on plasma samples. If both tests are positive on repeat analysis, a graft biopsy should be performed to establish a definitive diagnosis of BKN. It should be kept in mind that the positivity of the surrogate markers follows a dynamic pattern. In general, urine samples for decoy cells turn positive first, potentially followed weeks later by positive plasma PCR studies, before ultimately some weeks later, the histological diagnosis of BKN might be established.[11] In such a "window" period, a renal allograft biopsy may be initially negative for BKN before it is subsequently diagnosed in a repeat biopsy.[30] Thus, a patient positive for decoy cells and plasma PCR but without histological evidence of BKN is at high risk (> 50%) for developing subsequent viral disease. If BKN is diagnosed histologically, the patient should be treated (such as low-dose immunosuppression). However, in cases with concurrently diagnosed rejection, we choose to transiently treat the rejection episode first, before therapeutic attempts are made to treat BKN (the Basel "two-step approach"[26]). Because allograft dysfunction in these cases is due to BKN and rejection, significant improvement of graft function may not be seen until immunosuppression is lowered during the second step and virally induced ATN starts to heal. Although patients are being "treated" for BKN (low-dose immunosuppression), plasma PCR testing and screening for decoy cells are especially useful to *monitor* for viral clearance.[6,11,24,26,30] If both tests turn from positive to negative and remain negative on repeat testing, then one can safely assume that BKN has resolved (i.e., viral replication has

ceased[26]). Viral resolution may be further confirmed by a repeat graft biopsy. Eight of our 29 patients overcame BKN, on average, 115 days after the initial diagnosis of BKN (median; range = 57–1003 days). Using this approach for patient management, the duration of low-dose immunosuppression, which might trigger rejection episodes, can be optimized. Once BKN has been overcome, allografts can have a good long-term prognosis in cases with limited tubular atrophy and rejection-induced damage.[11,26] If grafts are lost due to BKN, retransplantation is an option. Although case numbers are low, reoccurrence of viral nephropathy in a second allograft has not been observed thus far (in 1 patient in Basel, a second one was reported[28]). A new transplant means a new round of risk factors and potential polyomavirus reactivation.

Addendum: Immunohistochemistry to Detect Polyomaviruses

(a) To detect polyomaviruses immunohistochemically, we use a mouse monoclonal antibody from Calbiochem/Oncogene, which gives very good results. The antibody detects the SV40 T antigen, which is common to all polyomaviruses (SV40, JC virus, and BK virus). For routine diagnostic workup, further subclassification of the different viral strains is generally not required.

Sometimes, control tissue that serves as a "positive staining control" is problematic. Possible options are (if well-documented cases of BKN are not available) as follows: (1) brain tissue from patients suffering from PML or (2) cytology preparations from the urine with decoy cells. Brain tissue (likely due to overfixation and excessive cross-linking) can sometimes give negative staining results; as an alternative, cytology preparations may be used.

(b) To detect the BK virus strain specifically, we use a mouse monoclonal antibody that is specifically directed against the BK virus–specific T antigen (Chemicon, catalog number: MAB 8505, clone name: BK-T.1). The antibody does not detect JC virus in the setting of PML. Immunohistochemistry can be performed on formalin-fixed, paraffin-embedded tissue sections (after trypsin pretreatment for 10-30 minutes, incubation of primary antibody at a dilution 1:10,000 at 4 °C overnight) on frozen material or cytology preparations. In our experience, the antibody can sometimes give some "nonspecific" background staining.

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References

1. Zeiler M, Hirsch HH, Prince O, Dalquen P, Dickenmann M, Mihatsch MJ, et al. Latency and activation of polyomaviruses [abstract]. **J Am Soc Nephrol 2001;12:953A**.
2. Kahan AV, Coleman DV, Koss LG. Activation of human polyomavirus infection-detection by cytologic technics. **Am J Clin Pathol 1980;74:326-32**.
3. Markowitz R-B, Thompson HC, Mueller JF, Cohen JA, Dynan WS. Incidence of BK virus and JC virus in human immunodeficiency virus-infected and -uninfected subjects. **J Infect Dis 1993;167:13-20**.
4. Sundsfjord A, Flaegstad T, Flo R, Spein AR, Pedersen M, Permin H, et al. BK and JC viruses in human immunodeficiency virus type 1-infected persons: prevalence, excretion, viremia, and viral regulatory regions. **J Infect Dis 1994;169:485-90**.
5. Koss LG. Inflammatory processes within the lower urinary tract. In: Diagnostic cytology and its histopathologic bases. Vol. 2, 4th ed. **Philadelphia: J. B. Lippincott; 1992. p. 909-13**.
6. Nickeleit V, Hirsch HH, Binet IF, Gudat F, Prince O, Dalquen P, et al. Polyomavirus infection of renal allograft recipients: from latent infection to manifest disease. **J Am Soc Nephrol 1999;10:1080-9**.
7. Randhawa PS, Finkelstein S, Scantlebury V, Shapiro R, Vivas C, Jordan M, et al. Human polyoma virus-associated interstitial nephritis in the allograft kidney. **Transplantation 1999;67:103-9**.
8. Baksh FK, Finkelstein SD, Swalsky PA, Stoner GL, Ryschkewitsch CF, Randhawa P. Molecular genotyping of BK and JC viruses in human polyomavirus associated interstitial nephritis after renal transplantation. **Am J Kidney Dis 2001;38:354-65**.
9. Andrews C, Shah KV, Rubin R, Hirsch M: BK papovavirus infections in renal transplant recipients: contribution of donor disease. **J Infect Dis 1982;145:276**.
10. Binet I, Nickeleit V, Hirsch HH, Prince O, Dalquen P, Gudat F, et al. Polyomavirus disease under new immunosuppressive drugs: a cause of renal graft dysfunction and graft loss. **Transplantation 1999;67:918-22**.
11. Nickeleit V, Hirsch HH, Zeiler M, Gudat F, Prince O, Thiel G, et al. BK virus nephropathy in renal transplants-tubular necrosis, MHC-class II expression and rejection in a puzzling game. **Nephrol Dial Transplant 2000;15:324-32**.
12. Randhawa P, Baksh F, Aoki N, Tschirhart D, Finkelstein S. JC virus infection in allograft kidneys. **Transplantation 2001;71:1300-3**.
13. Smith RD, Galla JH, Skahan K, Anderson P, Linnemann CC, Ault GS, et al. Tubulo-interstitial nephritis due to a mutant polyomavirus BKV strain, BKV (CIN), causing end-stage renal disease. **J Clin Microbiol 1998;36:1660-65**.
14. Randhawa PS, Khaleel-Ur-Rehmann K, Swalsky PA, Vats A, Scantlebury V, Shapiro R, et al. DNA sequencing of viral capsid protein VP-1 region in patients with BK virus interstitial nephritis. **Transplantation 2002;73:1090-4**.
15. Chen CH, Wen MC, Wang M, Lian JD, Wu MJ, Cheng CH, et al. A regulatory region rearranged BK virus is associated with tubulointerstitial nephritis in a rejected renal allograft. **J Med Virol 2001;64:82-8**.

16. Boldorini R, Zorini EO, Fortunato M, Bernardi M, Suono A, Benigni E, et al. Molecular characterization and sequence analysis of polyomavirus BKV strain in a renal allograft recipient. **Hum Pathol 2001;32:656-9**.
17. Purighalla R, Shapiro R, McCauley J, Randhawa P. BK virus infection in a kidney allograft diagnosed by needle biopsy. **Am J Kidney Dis 1995;4:671-3**.
18. Pappo O, Demetris AJ, Raikow RB, Randhawa PS. Human polyoma virus infection of renal allografts: histopathologic diagnosis, clinical significance, and literature review. **Mod Pathol 1996;9:105-9**.
19. Mathur VS, Olson JL, Darragh TM, Yen TSB. Polyomavirus induced interstitial nephritis in two renal transplant recipients: case reports and review of the literature. **Am J Kidney Dis 1997;29:754-8**.
20. Drachenberg CB, Beskow CO, Cangro CB, Bourquin PM, Simsir A, Fink J, et al. Human polyoma virus in renal allograft biopsies: morphological findings and correlation with urine cytology. **Hum Pathol 1999;30:970-7**.
21. Howell DN, Smith SR, Butterly DW, Klassen PS, Krigman HR, Burchette JL Jr, et al. Diagnosis and management of BK polyomavirus interstitial nephritis in renal transplant recipients. **Transplantation 1999;68:1279-88**.
22. Binet I, Nickeleit V, Hirsch HH. Polyomavirus infections in transplant recipients. **Curr Opin Org Transplant 2000;5:210-6**.
23. Hurault de Ligny B, Etienne I, Francois A, Toupance O, Buchler M, Touchard G, et al. Polyomavirus-induced acute tubulo-interstitial nephritis in renal allograft recipients. **Transplant Proc 2000;32:2760-1**.
24. Ramos E, Drachenberg CB, Papadimitriou JC, Hamze O, Fink JC, Klassen DK, et al. Clinical course of polyoma virus nephropathy in 67 renal transplant patients. **J Am Soc Nephrol 2002;13:2145-51**.
25. Ahuja M, Cohen EP, Dayer AM, Kampalath B, Chang CC, Bresnahan BA, et al. Polyoma virus infection after renal transplantation. **Transplantation 2001;71:896-9**.
26. Mayr M, Nickeleit V, Hirsch HH, Dickenmann M, Mihatsch MJ, Steiger J. Polyomavirus BK nephropathy in a kidney transplant recipient: critical issues of diagnosis and management. **Am J Kidney Dis 2001;38:E13**.
27. Elli A, Banfi G, Fogazzi GB, Tarantino A, Ponticelli C. BK polyomavirus interstitial nephritis in a renal transplant patient with no previous acute rejection episodes. **J Nephrol 2002;15:313-6**.
28. Poduval RD, Meehan SM, Woodle ES, Thistlethwaite JR, Haas M, Cronin DC, et al. Successful retransplantation after renal allograft loss to polyoma virus interstitial nephritis. **Transplantation 2002;73:1166-9**.
29. Limaye AP, Jerome KR, Kuhr CS, Ferrenberg J, Huang ML, Davis CL, et al. Quantitation of BK virus load in serum for the diagnosis of BK virus associated nephropathy in renal transplant recipients. **J Infect Dis 2001;183:1669-72**.
30. Nickeleit V, Klimkait T, Binet IF, Dalquen P, DelZenero V, Thiel G, et al. Testing for polyomavirus type BK DNA in plasma to identify renal allograft recipients with viral nephropathy. **N Engl J Med 2000;342:1309-15**.
31. Drachenberg RC, Drachenberg CB, Papadimitriou JC, Ramos E, Fink JC, Wali R, et al. Morphological spectrum of polyoma virus disease in renal allografts: diagnostic accuracy of urine cytology. **Am J Transplant 2001;1:373-81**.
32. Nickeleit V, Zeiler M, Gudat F, Thiel G, Mihatsch MJ. Detection of the complement degradation product C4d in renal allografts: diagnostic and therapeutic implications. **J Am Soc Nephrol 2002;13:242-51**.
33. Vats A. Viral load monitoring in BK virus associated nephropathy. **Nephrol News Issues 2002;3:S17-9**.
34. Hirsch HH, Mohaupt M, Klimkait T. Prospective monitoring of BK virus load after discontinuing sirolimus treatment in a renal transplant patient with BK virus nephropathy. **J Infect Dis 2001;183:1669-72**.
35. Hirsch HH, Knowles W, Dickenmann M, Passweg J, Klimkait T, Mihatsch MJ, Steiger J. Prospective study of polyomavirus type BK replication and nephropathy in renal transplant recipients. **N Engl J Med 2002; 347:488-96.**
36. Atencio IA, Shadan FF, Zhou XJ, Vaziri ND, Villarreal LP. Adult mouse kidneys become permissive to acute polyomavirus infection and reactivate persistent infections in response to cellular damage and regeneration. **J Virol 1993;67:1424-32**.