

Viral renal allograft infections: diagnostic clues and pitfalls

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Many infections can affect immunosuppressed renal allograft recipients either as systemic events or organ limited diseases. Some infections are restricted to the allograft. Here I will discuss the most important, clinically and diagnostically challenging productive viral infections found in kidney transplants. Table 1 lists helpful diagnostic clues when evaluating allograft biopsies.

Table 1. Histologic changes induced by intra renal viral replication – diagnostic clues (modified from Singh HK, Nিকেleit V: Kidney Disease caused by Viral Infections. *Curr Diag Pathol* 10:11-21, 2004)

	Adenovirus	Polyomavirus	Cytomegalovirus	EBV-PTLD
<u>Viral Inclusion Types</u>				
○ Nuclear: ground glass, homogeneous	+++	++	±	-
○ Nuclear: with halo	± / +	± / ++	+++	-
○ Nuclear: uncommon (granular/ clumped)	±	+ / ++	-	-
○ Cytoplasmic	-	-	+	-
<u>Sites of Viral Replication</u>				
○ Tubular epithelial cells	+++	+++	+++	-
○ Endothelial cells	-	-	++	-
○ Mononuclear cells	±	-	+	+++
<u>Acute Tubular Injury</u>	+++	± / +++	+ / ++	±
<u>Focal Parenchymal Necrosis</u>	± / +++	-	+	±
<u>Interstitial hemorrhage</u>	+ / ++	-	-	±
<u>Granuloma formation</u>	+ / ++	- / +	+	-
<u>Interstitial inflammation</u>	++	+ / ++	+ / ++	+++

A. Polyoma-BK-virus nephropathy (PVN)

1. Background

Polyomavirus allograft nephropathy (PVN) post kidney transplantation was first described nearly 3 decades ago in a kidney transplant recipient by the pathologist Mackenzie [1]. It was, however, not until the introduction of potent third generation immunosuppressive drugs, mainly high dose tacrolimus and mycophenolate mofetil, into clinical practice that transplant centers experienced “an epidemic” of PVN as a mostly iatrogenic complication due to “over immunosuppression” [2-8]. Specific risk factors of disease are, however, only poorly defined [9, 10]. Currently PVN has a prevalence of 1% to 10% and a reported graft failure rate of more than 50% in some series [7, 11-16]. Likely, the incidence of PVN will decrease in the future under altered and optimized immunosuppression. A recent encouraging report from the Mayo Clinic found a highly significant reduction of the incidence of PVN from 10.5% to 2.5% following the clinical introduction of “low dose” maintenance tacrolimus immunosuppression [7]. However, at present PVN still constitutes a major clinical challenge. Specific and potent antiviral drugs to treat productive polyomavirus infections are not available. Consequently, much emphasis is placed on patient screening and a diagnosis of PVN in an early disease stage that often responds favorably to our limited therapeutic options mainly consisting of a reduction of maintenance immunosuppression. Pathologists play a crucial role in risk assessment, diagnosis, and patient monitoring.

Infections with polyomaviruses and PVN are characterized by several key features:

- 1) PVN is caused by the re-activation of latent intra-renal/intra-graft, i.e. donor derived, polyomaviruses under long-lasting and intense immunosuppression.
- 2) Slight changes in the immune status can lead to transient, asymptomatic, and self-limiting activation of latent polyomaviruses [17], especially in the urothelium, which harbors latent BK virus infections in 43% of individuals [18]. Such activation is characterized by the detection of free viral particles in the urine (by electron microscopy or PCR techniques) and viral inclusion bearing cells, so-called “decoy cells” in urine cytology specimens. Signs of transient, asymptomatic viral activation can occasionally also be detected in serum samples by PCR. Such polyomavirus (re)activation is commonly not accompanied by PVN. PVN, on the other hand, is always associated and typically preceded by the activation of polyomaviruses [19-25].
- 3) PVN is typically diagnosed 10-14 months post transplantation with only anecdotal cases reported as early as 6 days or as late as 6 years post grafting [14, 26]. Depending on the extent of virally induced tubular injury, patients clinically present with varying degrees of allograft dysfunction.
- 4) PVN is best diagnosed histologically in a graft biopsy, which additionally provides prognostically relevant information on the disease stage or concomitant rejection. Since PVN affects the kidney in a focal fashion, adequate samples are needed to guarantee an optimal diagnostic yield. The diagnosis may be missed in 25% - 37% of cases if only one small core of renal cortex is sampled [13, 27]; occasionally multiple

step sections have to be studied with ancillary techniques (immunohistochemistry, in-situ hybridization) in order to establish a definitive diagnosis [28].

- 5) PVN is nearly always caused by a productive infection with the BK-virus strain. Only a minority of cases (approximately 20% - 30%) show activation of polyoma-BK- and JC-viruses simultaneously with, as yet, undetermined biological significance [28-30]. Polyomavirus nephropathies that are only induced by a productive JC-virus infection are rare and SV-40 virus infections are exceptional [28, 31, 32]. Morphologic changes induced by productive BK-, JC, or SV-40 polyomavirus infections are identical; ancillary techniques such as immunohistochemistry, in-situ hybridization, or PCR are required for the exact identification of viral strains.
- 6) In general, PVN persists for months or even years (more than 2 years in one of our patients). Rapid disease resolution within 3 – 4 weeks is rare.
- 7) PVN is typically limited to the transplant and “the worst case scenario” is graft failure. Thus, patients are usually not at risk for a generalized infection.

The definitive diagnosis of PVN ideally requires a kidney biopsy and the detection of characteristic histologic changes.

2. Morphologic changes and diagnosis

PVN is defined as an intrarenal, productive polyomavirus infection involving the renal medulla and/or cortex with light microscopic *and/or* immunohistochemical *and/or* in-situ hybridization evidence of viral replication accompanied by varying degrees of parenchymal damage ranging from minimal to marked.

Histology: Histologic signs caused by a productive polyomavirus infection are characteristically found in epithelial cells lining collecting ducts, tubules, and Bowman’s capsule (parietal epithelial cells). Viral replication and the assembly of daughter virions result in the formation of intra-nuclear inclusion bodies, cell injury, and lysis (Figure 1) [3, 5, 20, 21, 23]. The replication of polyomaviruses does not induce any histologically discernible cytoplasmic inclusions. Four types of virally induced nuclear changes have been described (types 1-4), hybrid forms are common [23, 33, 34]. Rarely (in my experience in approximately 5% of PVN cases) virally induced nuclear changes are absent, and viral replication can only be demonstrated by immunohistochemistry or in-situ hybridization (representing very early phases of polyomavirus replication; disease stage A). PVN typically involves renal tubules and collecting ducts in a focal fashion. Severely injured tubules containing many inclusion bearing epithelial cells are typically located adjacent to normal ducts. Viral inclusion bodies, especially in disease stage A, are often most abundant in the renal medulla. Glomerular capillary tufts, blood vessels, inflammatory and mesenchymal cells are typically non-permissive for the replication of BK-/polyomaviruses.

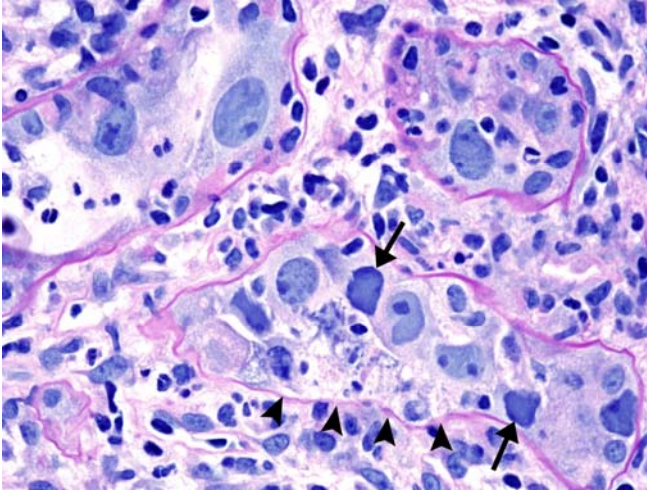


Figure 1: PVN, disease stage B

Tubules are markedly injured and contain epithelial cells with type 1 intranuclear viral inclusion bodies (arrows). Segments of tubular basement membrane (arrow heads) are denuded.

Uncommon histologic features of PVN include: pseudo-crescents in glomeruli (due to marked, virally induced injury of parietal epithelial cells [33, 35, 36]), non-necrotizing small epithelioid granulomas in and adjacent to severely injured tubules [37], and immune complex type deposits in tubular basement membranes [38]. These changes do not appear to carry any independent prognostic significance exceeding the staging of PVN (see below).

Viral inclusion bodies are also found in the transitional cell layer lining the renal pelvis, the (graft) ureter and potentially even the recipient's urinary bladder [33, 39]. Polyomavirus replication in the urothelium, however, is not a defining histologic feature characterizing PVN since it can also be seen in patients without viral nephropathy as a transient and asymptomatic sign of viral activation –or- in the setting of hemorrhagic cystitis (without PVN) following bone marrow transplantation [40, 41].

Ancillary diagnostic techniques: In tissue specimens, polyomavirus replication is readily detected with commercially available antibodies directed against the SV-40 T antigen (large T antigen) that cross-react with all polyomaviruses pathogenic in humans (i.e. BK-, JC-, and SV-40) and give a crisp, purely intra-nuclear staining signal. The expression of the T antigen marks the initial phase of polyomavirus replication and precedes the formation of intra-nuclear viral inclusion bodies in some infected cells. Thus, intense staining signals may be seen in normal nuclei/tissue [4, 23, 33, 42] as the earliest morphologic evidence of viral replication and PVN. Epithelial cells with signs of polyomavirus replication express p53 tumor suppressor proteins [43] and the proliferation marker KI-67 as signs of altered (viral) DNA assembly.

PCR techniques may also be utilized to demonstrate viral DNA or RNA in tissue samples and to confirm the diagnosis of PVN [44-46]. However, PCR results must be interpreted with great caution. Only very strong amplification signals of viral DNA (greater than 10 viral copies per cell equivalent), in the setting of histologically or immunohistochemically demonstrable virally induced cytopathic changes, can be used to confirm the diagnosis of PVN, to identify the viral strain, and to distinguish clinically

significant productive from clinically insignificant latent polyomavirus infections [44, 45, 47-51].

PVN and virally induced tubular injury are not associated with marked and diffuse upregulation of MHC-class II (i.e. HLA-DR) in tubular epithelial cells or with the deposition of the complement degradation product C4d along peritubular capillaries [4, 12, 52, 53]. Both, the detection of C4d and tubular HLA-DR expression can help to establish a diagnosis of (concurrent) allograft rejection [54].

By electron microscopy polyomaviruses present as viral particles of 30 to 50 nm in diameter that occasionally form crystalloid aggregates [20, 24]. Polyomaviruses are ultrastructurally identified by size and their icosahedral capsid structure; polyomavirus strains cannot be distinguished. Virions are primarily found in the nucleus, rarely in the cytoplasm.

Morphologic staging of PVN (Table 2): Most important for the clinical presentation and course of PVN are the tubular changes as severe, virally induced tubular injury and the denudation of tubular basement membranes can not only cause dysfunction, i.e. virally induced acute tubular injury (Figure 1), but also lead to atrophy and fibrosis. Based on the degree of tubulo-interstitial damage, PVN is sub-grouped into four disease stages: early “A”, fully developed “B” (Figure 1), fibrosing “C”, and burnt-out “D” [13, 20, 27, 42, 55-58]. Pertinent to staging are the severity of virally induced epithelial cell injury, interstitial fibrosis, and tubular atrophy rather than the extent of interstitial inflammation. Progression from one disease stage to another can occur within few months [12, 57]. The burnt-out stage of PVN (stage D) following the resolution of viral replication demonstrates non-specific chronic changes falling into the spectrum of so-called “chronic allograft nephropathy”.

the tubular expression of MHC-class II (HLA-DR) and/or the deposition of the complement degradation product C4d along peritubular capillaries [4, 19, 26, 53, 54, 59, 60]. *Of note:* PVN stage A lacks a significant inflammatory cell infiltrate. If only focal signs of polyomavirus replication are detected without significant epithelial cell lysis (i.e. disease stage A) in the setting of marked interstitial inflammation, then PVN and a concurrent cellular tubulo-interstitial rejection episode (Banff category 4, type 1) has to be considered [54]. The immunohistochemical phenotyping of the inflammatory cells in PVN has shown plasma cell (CD138) as well as B (CD20) or T cell (CD3) dominant infiltrates with currently undetermined pathophysiological significance.

Immunophenotyping, i.e. the detection of CD20 (B cells) versus CD3 (T cell) rich lymphocytic infiltrates, is diagnostically not helpful for distinguishing viral nephritis from concurrent acute allograft rejection [54, 61-63]. If concurrent rejection is diagnosed in the setting of PVN, anecdotal reports indicate that patients benefit from transient anti-rejection therapy before, in a second phase, therapeutic attempts are initiated to treat PVN [19, 23, 34, 54, 56, 64].

3. Clinical screening assays

The optimal timing of a diagnostic transplant biopsy becomes a clinical challenge, in particular in disease stage A that can present with stable graft function [10, 65]. Overall improved graft survival has been reported from centers with vigorous patient screening programs that facilitate an early intervention [19, 23, 34, 56, 64, 66-68].

Renal allograft recipients at risk for PVN and in whom a diagnostic graft biopsy is indicated can be clinically identified by detecting signs of polyomavirus activation. Commonly used “BK-virus-activation-assays” include: quantitative PCR tests (best performed on plasma or alternatively urine samples), urine cytology, and urine electron microscopy [4, 25, 56, 57, 67]. Patients with PVN typically present with large numbers of polyomavirus inclusion bearing epithelial cells in the urine, i.e. so-called decoy cells (10 or more decoy cells per ThinPrep slide; positive and negative predictive values for PVN: 27% and 100%, respectively) [2-4, 19, 24, 33, 34, 57, 69, 70]. Similar predictive values are found for quantitative PCR assays on voided urine samples with cut-off levels of 1×10^7 BK virus copies/ml to classify test results as either “positive” or “negative”. All patients with viral nephropathy shed abundant free virions in the urine detectable by negative staining electron microscopy [6, 25, 71]. Among all “BK-virus-activation-assays” quantitative plasma PCR tests (cut-off 1×10^4 BK virus copies/ml) predict disease best with 74% probability (negative predictive value: 87%).

In Chapel Hill we often use an alternative, powerful, new, non-invasive method to accurately diagnose PVN in urine samples. The urine test is not based on general signs of BK-virus-activation but rather targets three dimensional, cast-like polyomavirus clusters as specific disease markers (Figure 2). Dense aggregates of polyomaviruses (so-called “Haufen”, a German term for “stack” or “heap”) form in virally injured tubules with Tamm-Horsfall protein similar to the development of red or white blood cell casts. The cast-like polyomavirus aggregates are flushed into the urine, and can easily be detected by standard, routine negative staining electron microscopy in voided urine samples. Urinary Haufen, in sharp contrast to all BK-virus-activation-based-assays, are disease

specific. Their presence clearly marks intra renal disease, i.e. PVN (positive and negative predictive values: 97% and 100%, respectively) [72].

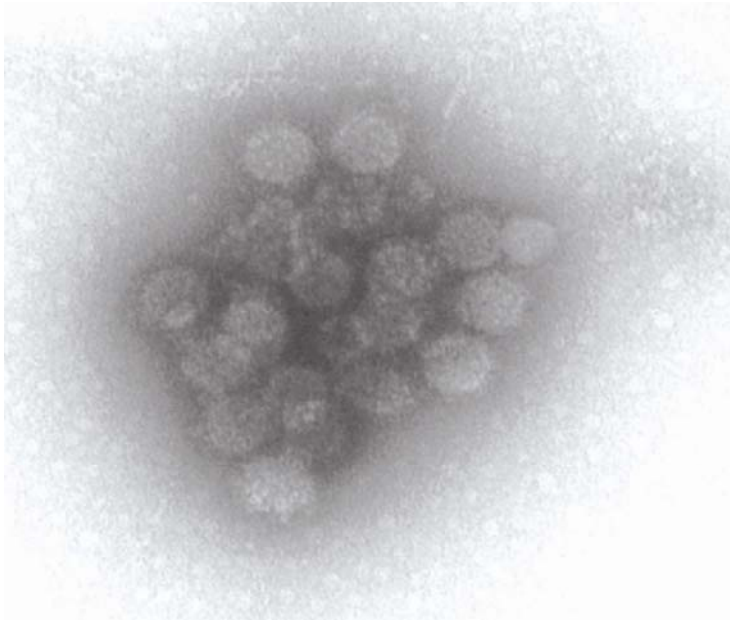


Figure 2: Voided urine sample with polyomavirus 'Haufen'

In cases of PVN cast-like, three dimensional polyomavirus aggregates, termed 'Haufen', are typically shed into the urine. Haufen form in virally injured tubules (compare with Figure 1) and are PVN disease specific. (*Negative staining electron microscopy, 100.000x magnification*)

Thus, pathologists can play a crucial role in the risk assessment for PVN. One voided urine sample can be analyzed by cytology (to search for decoy cells) and potentially also by negative staining electron microscopy (to search for Haufen; for technical details see [25]). If both tests are positive the diagnosis of PVN is established. A renal biopsy may still be obtained for disease staging and to rule out concurrent rejection.

In general all screening test results should be made available to histopathologists at time of a graft biopsy; positive tests should trigger an intensive search for morphologic signs of polyomavirus replication (including the analysis of multiple step sections and ancillary immunohistochemical or in-situ hybridization studies).

B. Adenovirus

Adenovirus infections of renal allografts are very infrequent complications post transplantation [73, 74]. Morphologic changes caused by productive infections are summarized in Table 1; they include: 1) intra nuclear viral inclusions in epithelial cells; 2) severe tubular destruction with rupture of basement membranes and focal necrosis; 3) marked interstitial inflammation; 4) focal interstitial hemorrhage and intra tubular red blood cell casts (Figure 3) [75, 76]. The replication of adenoviruses induces cytopathic intra nuclear changes in tubular cells and occasionally also in parietal epithelial cells lining Bowman's capsule with mostly smudgy, 'ground-glass' type of inclusion bodies (similar to polyomavirus type 1 changes). Rarely, viral inclusion bodies are surrounded

by a halo (i.e. CMV-like) [77, 78]. Nodular and granulomatous inflammatory cell infiltrates are found in areas with marked viral replication. They are primarily composed of mononuclear and plasma cells. Foci of necrosis and tubular disruption show abundant polymorphonuclear leukocytes. On occasion necrotic regions can be large and wedge shaped. Glomeruli and blood vessels are generally not affected. Common ancillary diagnostic techniques include immunohistochemistry and electron microscopy. Immunohistochemistry with antibodies directed against outer viral antigens shows strong nuclear and less intense cytoplasmic staining in epithelial cells. Ultrastructurally virions of approximately 75 – 80 nanometers are found in nuclei and the cytoplasm. Free viral particles can typically also be detected in the urine by negative staining electron microscopy. In contrast to PVN, however, dense viral aggregates, i.e. Haufen, or abundant decoy cells are not seen in voided urine samples. Immunofluorescence microscopy with a standard panel of antibodies directed against immunoglobulins and complement factors is unrevealing. In my experience adenovirus infections are not associated with the deposition of the complement degradation product C4d along peritubular capillaries.

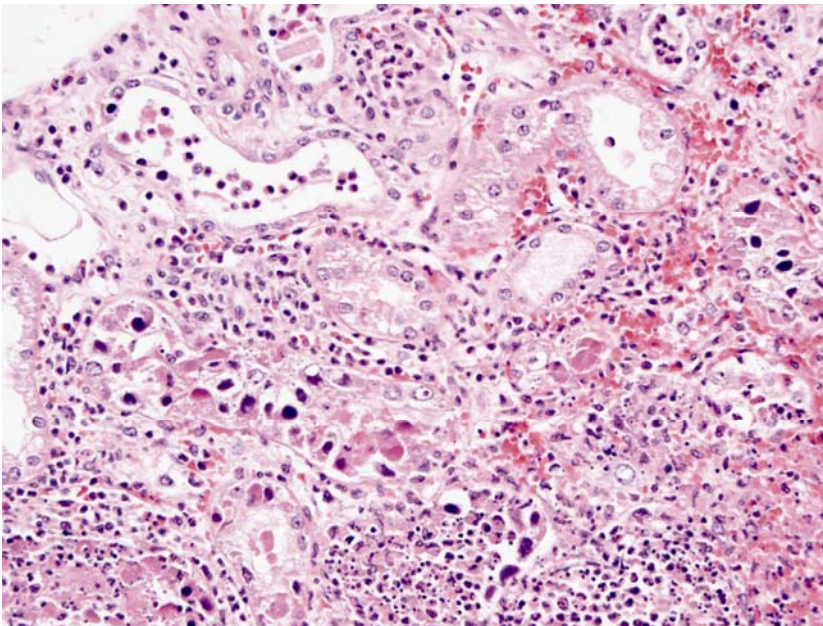


Figure 3: Adenovirus Infection

Tubules are severely injured and contain smudgy, intranuclear viral inclusion bearing epithelial cells. Typical for adenovirus infections are foci of interstitial hemorrhage, polymorphonuclear leukocytes, and necrosis (compare to Figure 1).

The differential diagnosis includes other types of viral infections, mainly PVN. Adenovirus replication can usually be suspected by light microscopy based on: a) frank tubular destruction with foci of necrosis; b) granulomatous inflammation with palisading of macrophages around severely injured tubules; c) interstitial hemorrhage. A superimposed second viral kidney infection in cases of adenovirus induced nephritis seems to be rare; in one series only 1/14 patients demonstrated concurrent focal PVN and 0/14 signs of CMV replication [78].

Adenovirus infections are most often caused by subgroup B, serotypes 7, 11, 34, and 35. Serotypes 34, 35 and most frequently 11 have been associated with hemorrhagic cystitis

and necrotizing interstitial nephritis [77-81]. Infections can be asymptomatic, cause localized disease such as enteritis, cystitis or nephritis, or present as disseminated severe illness. Patients suffering from adenovirus induced nephritis most often present within the first three months after transplantation with renal failure, hematuria, sometimes dysuria and hemorrhagic cystitis, and often (but not always) with evidence of a generalized infection including fever. Although treatment strategies for adenovirus allograft infections are not well defined (they include the reduction of the immunosuppression, IVIG, intravenous ribavirin, and ganciclovir [74, 79-83]), most patients nevertheless recover surprisingly rapidly with profound improvement of renal function and long-term graft survival [74, 80, 82-84]. In one patient viral clearance from the kidney transplant could be documented in a repeat graft biopsy within 4 weeks [81]. Disseminated adenovirus infections can be fatal [77, 78, 85-87].

C. Cytomegalovirus (CMV)

Cytomegalovirus (CMV), a herpesvirus, is one of the most common pathogens in renal transplant recipients. CMV can cause a symptomatic infection during the first months after transplantation, generally characterized by fever, leukopenia, hepatitis, or pneumonitis, and viremia [88]. The kidneys are infrequently affected in only approximately 25% of patients suffering from 'CMV disease' [89, 90]. In the western world effective patient screening and clinical management strategies have made productive CMV infections of renal transplants exceedingly rare. This observation contrasts a recent study from India reporting a prevalence of CMV renal allograft infections of 1.9% [91].

Lesions induced by the replication of CMV in the kidneys have been described both in native organs and transplants (Table 1 [89, 90]. Cytopathic changes are typically very focal and most often seen in the nuclei and cytoplasm of tubular epithelial cells, sometimes in endothelial cells, and only occasionally in mononuclear inflammatory cells [89, 90]. CMV infected cells are enlarged with nuclei containing a central round inclusion body surrounded by a circumferential halo, i.e. the typical "owls-eye" appearance. Also homogenous smudgy appearing intranuclear inclusions are occasionally observed. Small basophilic "lumpy" cytoplasmic viral inclusions are frequently (but not always) detected in cells with virally induced intranuclear changes [89]. The replication of CMV in the tubular compartment or endothelial cells in peritubular capillaries can be associated with a nodular, sharply circumscribed and occasionally granulomatous appearing mononuclear inflammatory cell infiltrate. Interstitial inflammation was absent in 2/6 cases of CMV nephropathy in one series [90]. Foci of necrosis and micro abscesses can occur, but are uncommon [90]. Rarely, CMV infects glomerular cells, and causes an acute glomerulonephritis with crescents [92-95]; in one exceptional case CMV replication was limited to glomerular endothelial cells and not accompanied by inflammation [94].

Ancillary diagnostic techniques to confirm the diagnosis of a productive CMV infection include immunohistochemistry (e.g. best with an antibody directed against the immediate early antigen), in-situ hybridization or by electron microscopy. Ultrastructurally virions of approximately 150 nanometers are found in nuclei and the cytoplasm. Immunofluorescence microscopy with a standard panel of antibodies detecting immunoglobulins and complement

factors is unrevealing [89]. Whether a productive intra graft CMV infection is associated with the accumulation of the complement degradation product C4d along peritubular capillaries is unknown, but it seems to be unlikely.

CMV DNA is found in the absence of cytopathic changes, and consequently, PCR studies for viral DNA do not clearly distinguish between productive and latent infections [96]. Thus, it seems doubtful whether the use of highly sensitive techniques (such as tissue CMV-PCR) really demonstrates a higher prevalence of “CMV disease” as suggested by some [97]. The minimal criteria to establish the diagnosis of CMV nephritis include the demonstration of cytopathic changes, or CMV proteins, or mRNA. This diagnostic strategy is not unique to CMV infections but similar to other potentially pathogenic DNA viruses that establish latency in humans, such as polyomaviruses (see above). The differential diagnosis of CMV nephropathy includes other types of viral infections, mainly caused by polyomaviruses or adenovirus. Since CMV (in contrast to polyomavirus and adenovirus) often replicates in endothelial and inflammatory cells, a distinction between rejection induced changes and infection driven inflammation is difficult. Some original reports had described a “CMV-glomerulopathy” [98]; this lesion is now classified as rejection induced transplant glomerulitis.

CMV infections can stimulate indirect effects on the kidney graft by modulating the immune response and promoting rejection episodes [88]. The most convincing evidence that CMV indirectly causes graft injury was reported by Reinke, who showed that 85% of patients with "late-acute rejection" responded to ganciclovir therapy [99].

D. Epstein Barr Virus / post transplant lymphoproliferative disorders

1. Background

According to the 2001 WHO definition “post transplant lymphoproliferative disorder is a lymphoid proliferation or lymphoma that develops as a consequence of immunosuppression in a recipient of a solid organ or bone marrow allograft. PTLDs comprise a spectrum ranging from early, Epstein-Barr virus (EBV) driven polyclonal proliferations resembling infectious mononucleosis to EBV positive or EBV negative lymphomas of predominately B-cell or less often T-cell type” (Table 3) [100]. Molecular studies have shown a progression from polymorphism, to clonality and subsequent oncogene mutations [101][102]. A prerequisite for the development of PTLD is intense immunosuppression targeting T cells. The higher the dose, the more rapid the onset of PTLD, which can arise as soon as one month post-transplantation. The intensity of the immunosuppression probably also accounts for the difference in the prevalence of PTLD in heart and kidney transplant recipients. The risk for developing lymphoid neoplasias is estimated to be 20 times that of the normal population for renal allograft and 120 times normal for cardiac transplant recipients [100]. Another major risk factor is the constellation of the recipient’s and donor’s EBV serostatus, i.e. transplantation from a sero-positive donor into a sero-negative recipient is associated with a high risk for PTLD [103].

PTLD (limited for study purposes to cases of ‘developing non-Hodgkin lymphomas’) has been reported with a prevalence of 1.4% among 25,127 renal allograft recipients transplanted in the US in the late 1990s [104], a similar prevalence was reported from the transplant center in Pittsburgh (1.2% in adults and 10.1% in pediatric kidney transplant recipients on tacrolimus therapy[105]). PTLDs may be less common in other parts of the world [106].

Table 3. Post-Transplant Lymphoproliferative Disease (PTLD)*

Early Lesions
Reactive plasmacytic hyperplasia
Infectious mononucleosis-like
PTLD-Polymorphic
Poly-/ oligoclonal (rare)
Monoclonal
PTLD-Monomorphic
B-cell neoplasms
- Diffuse large B-cell lymphoma (<i>immunoblastic, centroblastic, anaplastic</i>)
- Burkitt's/Burkitt's-like lymphoma
- Plasma cell myeloma
- Plasmacytoma like B-cell lymphoma
T-cell neoplasms
- Peripheral T-cell lymphoma (NOS)
Other
Hodgkin and Hodgkin like lymphoma

WHO classification of PTLD

(from: *Harris NL, Swerdlow SH, Frizzera G, et al.: Post-transplant lymphoproliferative disorders, in Pathology and genetics of tumours of haematopoietic and lymphoid tissues edited by Jaffe ES, Harris NL, Stein H, Vardiman JW, Lyon, IARC Press, 2001, pp 264-269*)

Overall more than 80% of the tested PTLD cases are EBV positive [100, 107, 108]. EBV negative cases are more frequent among renal allograft recipients presenting with T cell lymphomas [100, 109]. B-cells are the origin of more than 85% of PTLDs in organ transplant recipients [110, 111]. In contrast to the ordinary B cell lymphomas, clonality of the neoplastic cells is not always demonstrable, in particular in ‘early’ and ‘polymorphic’ PTLD variants. T cell lymphomas comprise approximately 15% and null cells <1% of PTLDs [110]. The majority (90%) of PTLDs in solid organ recipients are of host origin [103]; only rare PTLDs arise from transplanted donor lymphocytes.

In general, EBV positive PTLDs arise within the first two post transplant years, whereas EBV negative cases have a median onset of 50 – 60 months after grafting [112, 113]. PTLD rarely involves the peripheral blood, but rather extranodal sites. The distribution of EBV positive PTLDs was documented in a series of 9 non-human primate research animals with kidney transplants that showed involvement of lymphnodes (100%

of cases), liver (56%), lung (44%), heart (44%), renal allograft (44%), and native kidneys (22%) [114]. In humans PTLDs involve the renal allograft in approximately 14% - 30% of patients [106, 115, 116]; they were restricted to the kidney transplant in 12% of cases in one series [116]. PTLDs restricted to the kidney transplant often occur early (on average 5 months) post surgery, are more frequently of donor cell origin and fare favorably [103]. The overall 5-year adult patient and graft survival in PTLD was 86% and 60% in Pittsburgh [105]; patient survival was reportedly less favorable in high grade, lymphomatous PTLD variants [103, 104]. In particular polymorphic PTLDs respond to antiviral therapy and a reduction in immunosuppression, whereas monomorphic, monoclonal PTLDs that show mutations of the Bcl 6 gene (seen in approximately 90% of cases) require specific anti-tumor therapy [103]. More recently successful therapeutic attempts were made with the anti CD20 antibody rituximab in patients with monomorphic and polymorphic PTLDs resulting in complete remission in more than 50% of patients [117, 118]. PTLDs of T-cell lineage have a poor prognosis [103].

The clinical presentation is heterogenous and dependent upon the location and extent of the disease. Patients with kidney transplant involvement typically present with graft dysfunction and may sometimes show a “mass lesion” in imaging studies. The histologic diagnosis of PTLD in a renal allograft is challenging, in particular, in unsuspected cases limited to the transplant.

2. Morphologic changes and diagnosis

Histology: Most cases of PTLD involving the kidneys are of the polymorphic variant [115, 116] and can, therefore, mimick cellular rejection. The interstitial compartment typically shows vaguely nodular, expansile aggregates of mononuclear cell elements containing plasma cells and varying numbers of activated lymphocytes admixed with ‘blastoid’ cell elements containing prominent nucleoli (Figure 4). Mitotic figures can usually be found and occasionally foci of serpiginous necrosis. The neoplastic mononuclear cells often invade tubules (i.e. tubulitis); they can also penetrate into arterial intimal layers (i.e. transplant endarteritis). Transplant glomerulitis, however, is uncommon. The replication of EBV does not induce any viral inclusion bodies.

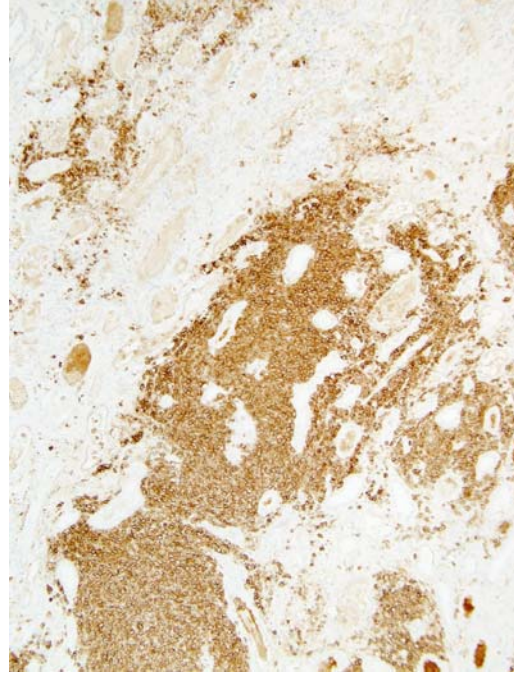
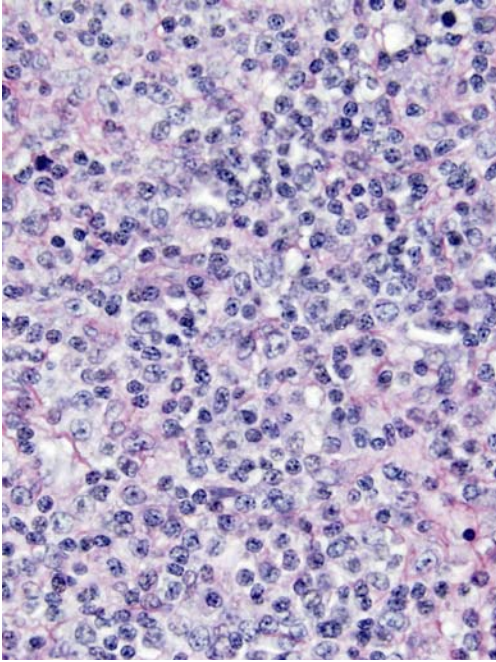


Figure 4: PTLD

PTLD is characterized by expansile infiltrates of activated lymphocytes containing blastoid cell elements. Interstitial edema is usually inconspicuous. Immunohistochemistry shows abundant CD20 positive cells.

The major differential diagnosis of PTLD involving renal allografts is acute rejection [108, 121] that would be treated with an increase in immunosuppression. Both in rejection and PTLD the mononuclear cells have enlarged nuclei with nucleoli, and they can show mitotic activity (although ‘blasts’ and mitoses are more common in PTLD). In addition tubulitis and endarteritis may be found in PTLD. Several features should raise the suspicion of PTLD. The most helpful clue in my experience is the presence of dense, vaguely nodular, expansile sheets of activated lymphoid cell elements without an admixture of granulocytes or macrophages. Tubules often seem to be “pushed apart”. In comparison to cellular rejection that typically presents with conspicuous interstitial edema, neoplastic infiltrates in PTLD are commonly rather densely packed with only minimal edematous fluid. PTLDs, but not cellular rejection, commonly involve the renal capsule and perirenal adipose tissue. The presence of serpiginous necrosis is also a sign of PTLD [116], although its absence is not helpful, nor is interstitial hemorrhage that can be seen in severe rejection episodes. To make things worse, it has been suggested that rejection can co-exist with PTLD [116].

Ancillary diagnostic techniques: Immortalized B cells express six nuclear antigens (e.g. EBNA-2), two EBV-encoded nuclear RNAs (EBERs) and three membrane proteins (e.g., latent membrane protein, LMP-1) that are of diagnostic value during the histologic work-

up of renal biopsies (reviewed in [125]). Since the vast majority of PTLDs is of B cell lineage and driven by the replication of EBV diagnostic confirmation can generally be achieved by immunohistochemistry (demonstrating abundant CD20 –Figure 4, CD19 and/or CD 79a positive cells of B lineage) as well as in-situ hybridization for EBER (revealing strong and diffuse staining signals; the detection of rare EBER positive cells should not be considered diagnostic for PTLD [100, 123]). CD3 and CD68 positive cells, hallmarks of cellular rejection, are generally relatively sparse, i.e. accounting for less than 50% of the inflammatory cell infiltrates. In contrast to cellular rejection, in PTLD B-cells are also found in foci of tubulitis and endothelialitis. Immunohistochemical stains for LMP-1 and usually EBNA can be focal and scattered making them less reliable markers in the diagnostic decision making process. In a systematic study of 14 cases of PTLD [125], EBER was detected in all cases by in situ hybridization; 11 were positive for LMP-1 and 9 for EBNA-2 by immunohistochemistry. The number of positive cells was highly variable, even within the same tumor; positivity was increased around areas of necrosis. Tests to detect clonality (e.g. immunoglobulin gene rearrangement) help to confirm the presence of a monomorphic PTLD; polyclonality, however, does not exclude early and polymorphic PTLD variants [120]. Additional clinical information (such as preceding rejection episodes treated with ATG or OKT3) and significantly elevated plasma EBV viral load levels (by PCR) may further help to confirm the diagnosis of PTLD. Rare forms of T-cell mediated PTLDs may cause diagnostic problems since they are often EBER negative [100]; these latter tumors, however, commonly present as high grade neoplasms that can be easily distinguished from acute cellular rejection.

References

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