of rejection (kappa 0.72) and the presence or absence of endarteritis (kappa 0.65). The design of this study did not intrinsically favor agreement, in that the scoring was done over several years by almost a dozen pathologists. Thus, the CCTT classification system appears to be statistically robust. The original Banif system compares unfavorably with CCTT in kappa values, It is obviously difficult to compare kappa values between these two studies, however, it is notable that the agreement rate for endarteritis was equivalent in the two studies. Since this feature is defined similarly by the two groups, this result suggests that the other marked differences in agreement are intrinsic to the classification schemes rather than the design of the studies or the skills of the pathologists.

The pathologists in this study found that the CCTT classification of the biopsies takes no more time than the usual diagnostic examination of a transplant biopsy. In contrast to Banff, little has to be quantitated: the estimated percentage of cortex involved with the infiltrate has to exceed 5% and the tubulitis occasionally has to be counted to be certain that at least three tubules are affected. Nothing else is graded: there are no "mild", "moderate" or "severe" degrees of any lesions. As a measure of the efficiency, the review panels typically took about 5 min per biopsy.

The CCTT criteria have a sensitivity of 90% for detection of rejection in one core and a calculated sensitivity of 99% for two cores, which is quite satisfactory for clinical management. The specificity of the pathological criteria is difficult, if not impossible, to determine since the biopsy is widely regarded as the "gold standard". When rejection was defined solely by clinical criteria and the biopsy was interpreted without any clinical information, the CCTT classification performed acceptably, with a sensitivity of 86% and a specificity of 72%. When judged by the clinical course, a significant fraction of patients who lack clinical evidence of rejection (28-38%) have a biopsy that meets the pathological criteria for rejection (4). It must be considered possible, if not likely, that these discrepancies are not due to a lack of specificity of the biopsy criteria, but rather that rejection is subclinical. Past published data do not support prognostic significance of the extent of the infiltrate or tubulitis, even if it could be accurately graded (1, 2). When the diagnostic criteria for the number of tubules with tubulitis and the percent infiltrate were varied, the greatest agreement with a clinical course consistent with rejection were using the original criteria, validating the thresholds set. Banif has a higher threshold of infiltrate for Grade I rejection (25% vs. 5%). Our results indicate that the infiltrate involves less than 25% of the cortex in 31% of the cases of Type I rejection. Thus, CCTT would classify many of the Banif borderlines as a Type I rejection. The addition of the requirements for edema, activated lymphocytes or tubular injury made no difference in diagnostic accuracy. However, 90% of these biopsies were for graft dysfunction, and the criteria may be useful for protocol biopsies.

The CCTT types of rejection correlate with clinical severity. Type I rejection is more often completely steroid responsive (5). Type II rejection was six times more likely to be clinically severe than Type I (1), confirming several previous studies noted above that have suggested that endarteritis has adverse prognostic significance. Type III rejection has a well-known adverse prognostic significance in all series. Hemorrhage and glomerulitis also are adverse prognostic features. Thus, the CCTT system has certain objective and major advantages, notably simplicity and reproducibility, while retaining sensitivity, specificity and clinically relevant prognostic implications.

The revised Banff classification recognizes the validity of the CCTT system and uses the same major categories ("borderline" has become "suspicious"). Major remaining issues for the future are to define more markers for active rejection versus harmless (?beneficial) infiltration in an accepted graft and to incorporate acute humoral rejection and glomerular lesions into the system.

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Polyomavirus infection of renal allografts

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A morphologically manifest *Polyomavirus* infection of renal allografts with the BK-virus strain is a new and highly unusual complication carrying an unfavorable prognosis. *Polyomavirus,* a subgroup of the papovavirus family, is a double-stranded nonencapsulated DNA virus. After a mostly asymptomatic primary infection early in life, *Polyomavirus* frequently resides in a dormant state in the kidneys and ureters of healthy individuals. In immunocompetent hosts, it does not cause symptomatic disease. On the other hand, immunocompromised patients are at risk of a clinically manifest infection. Human disease can be caused by two *Polyomavirus* strains: JC and BK. JC-virus is the causative agent of progressive multifocal leukoencephalopathy. BK-virus is associated with changes in the kidney and the urothelium, *i.e.,* "viral nephritis" and, proposed by some, hemorrhagic cystitis. However, a clinically symptomatic *Polyomavirus* infection is exceptional, even under immunosuppression. The kidney, a common site of dormant viruses, is hardly ever affected. In Basel we did not encounter a single case of a manifest renal allograft infection with *Polyomavirus* before 1996, whereas nine cases were diagnosed in the following 3 years, which points to new risk factors (1).

Polyomavirus disease

Polyomavirus disease defines a histologically manifest renal allograft infection with viral inclusion bodies, associated with rather varying degrees of interstitial inflammation and deterioration of graft function *(i.e.,* an increase in serum creatinine). The initial diagnosis is made months after transplantation (range: 4-25 months) (2, 3). In man, the disease is caused by the BK-virus strain (2).

The diagnosis of *Polyomavirus* disease is only made histologically in a graft biopsy (2). The morphological hallmark is the detec-

tion of intranuclear viral inclusions, which are exclusively found in epithelial cells (with the exception of podocytes). Intranuclear inclusion bodies can be seen in four different types with similar frequencies along the entire nephron: i) an amorphous ground-glass variant; ii) a central, eosinophilic, granular type surrounded by a (mostly incomplete) halo; ii) a homogenous, finely granular form lacking a halo; and, iv) a vesicular type with enlarged nuclei and clumped, irregular chromatin. The latter (type 4) inclusion body is the least characteristic and often only distinguished after immunohistochemical incubations searching for *Polyomavirus.* Type 4 inclusions are primarily seen in grafts with longstanding disease. Cells with viral changes are often (but not always) enlarged and can have polymorphic nuclei, especially with type 4 inclusions. Frequently, tubular cells are rounded-up, necrotic and extruded from the epithelial cell layer into tubular lumens causing marked denudation of basement membranes. Intratubular "cellular" casts and denuded basement membranes are sometimes the first diagnostic clue on low power microscopic examination drawing the attention to an underlying viral infection. Although cytopathic signs are seen along the entire nephron, they are often abundant in distal tubular segments and collecting ducts. Sporadically, infected cells are noted in the parietal epithelium lining Bowman's capsule, occasionally even forming small crescents. In the renal pelvis and ureters, viral inclusion bodies are mainly seen in superficial transitional cells, rarely in the basal cell layer. Although the morphological changes are typical of an infection with polyomavirus, they are not pathognomonic. Herpes simplex virus, adenovirus and possibly even cytomegalovirus (CMV) have to be considered in the differential diagnosis. The latter viruses can easily be excluded by immunohistochemistry (employing an antibody to detect SV 40 large T antigen, i.e., "pan" *Polyoma virus* antigen) or electronmicroscopy (2). In our cases of *Polyomavirus* disease, we did not encounter a cointection with CMV, Epstein-Barr virus, herpes simplex virus, varicella or adenovirus (1, *2).*

If inclusion bearing cells are sloughed into the urine, they can easily be detected in cytological preparations as "decoy cells". Decoy cells are a characteristic and constant finding in *Polyomavirus* disease. They were excreted in all of our patients during the course of disease and preceded the histological diagnosis by months. However, the detection of decoy cells in a renal allograft recipient does not necessarily indicate *Polyoma virus* disease with renal parenchymal involvement. In general, decoy cells only indicate an asymptomatic activation of *Polyomavirus* which is normally fully reversible. Polymerase chain reaction (POR) is an inadequate tool for screening urine samples since the technique is by far too sensitive.

In our patients, BK-virus was constantly detectable in the serum during the course of *Polyomavirus* disease by PCR (4). Similar to the excretion of decoy cells, also viremia could precede a histologically manifest disease by months. Viremia was asymptomatic and not associated with hematogenous spread to organs outside the kidney/urothelium.

In *Polyomavirus* disease, graft function is impaired and graft survival decreased (45% graft loss) (1). Two factors contribute to functional impairment: i) tubular necrosis induced by *Polyomavirus* infection, and ii) interstitial and intimal fibrosis. *Polyomavirus* infection causes frank tubular necrosis and, thus, alters renal function significantly. Sloughed necrotic epithelial cells form intraluminal casts leaving behind denuded tubular basement membranes. Denuded areas of basement membranes permit leaking of fluid into the interstitial compartment which is associated with functional impairment. In addition, tubular casts may cause an obstructive component. Such pathways are not unique to *Polyomavirus* disease but are well described in other forms of acute tubular necrosis. Since *Polyomavirus* in our experience never cleared from the kidneys (even months after the initial diagnosis), tubular injury did not heal, and, therefore, functional impairment persisted. In addition, overtime recurrent rejection episodes with transplant endarteritis led to interstitial and intimal fibrosis (i.e., "chronic allograft rejection") (2).

Controversy exists whether the interstitial inflammatory cell infiltrate in *Polyoma virus* disease represents virally induced interstitial nephritis or cellular rejection (1-3). Our data strongly suggest that *Polyomavirus* does not stimulate a marked inflammatory response, since a manifest infection with intranuclear inclusion bodies is often associated with an inconsistent, randomly distributed and occasionally even scant inflammatory reaction which is mostly due to tubular injury/necrosis (1, 2). Tubules with viral inclusions do not upregulate major histocompatibility complex (MHC)-class II, and intercellular adhesion molecule-i (ICAM-1) is only sporadically and weakly expressed (1). Cellular rejection should be considered if abundant cortical mononuclear cell infiltrates and typical tubulitis are found, randomly affecting tubules with and without cytopathic signs. This interpretation is supported by the observation that tubulitis is seen in areas lacking virally infected cells. If typical signs of interstitial cellular rejection are present, tubules characteristically express MHC-class II and ICAM-1 (1) – phenomena well known to occur during rejection.

What triggers *Polyomavirus* disease

Polyoma virus disease is a new complication in renal allograft recipients. In a previous study (5) we stressed that new immunosuppressive drugs are major risk factors in stimulating disease, in particular protocols containing high dose tacrolimus. In a recent publication from Pittsburgh 20/22 patients (90%) with *Polyoma virus* disease were on tacroliinus (3). All of our patients suffered from biopsy-proven rejection episodes in the months preceding disease (2). All patients had BK viremia and excreted decoy cells in the urine (4, 5). Decoy cells and viremia preceded disease (1, 2, 4). Thus, we propose the following risk profile: i) activation of *Polyomavirus* with significant decoy cell excretion in the urine (asyinptoinatic, fully reversible); ii) long-lasting tubular injury rendering epithelial cells susceptible to the virus (such as rejection or schemia); ii) high dose immnunosuppression, frequently tacrolimus based; and, iv) heinatogenous viral spread. Several risk factors have to concur.

Conclusion

Polyomavirus disease of renal allografts caused by BK virus is a new complication. The diagnosis is only made in a graft biopsy. The detection of decoy cells in the urine and BK viremia are useful adjunct tools to screen high risk patients and to monitor the course of this disease. *Polyomavirus* disease causes impaired graft function, mainly due to protracted severe tubular injury/necrosis. At present, there is no specific antiviral therapy available. The only current therapeutic option is a decrease of maintenance immunosuppression, perhaps cyclosporine based, to promote clearance of the virus (1, 3, 6). This approach is granted, since *Polyomavirus* does not trigger marked inflammation or rejection (1). If rejection coincides with *Polyomavirus* disease, it can be diagnosed in a graft biopsy.

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Capillary C4d: A tool for the diagnosis of transplants at risk

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The occurrence of delayed graft function (DGF) together with early acute rejection, and the development of chronic rejection account for the majority of *graft* losses in recipients, Clinical and experimental observations suggest that acute transplant rejections, depending on their severity and type, have a strong impact on the long-term survival of renal allografts as they may be related to the development of chronic rejection. It is generally assumed that rejection episodes are dominated by T-cell mediated reactions since infiltrating lymphocytes within the interstitium and in vessel walls are easily detectable upon histopathological examination of graft biopsies. In contrast, the role of humoral immunity in transplantation remains ill-defined because humoral immune reactants are usually not detectable in graft biopsies and the manifestations of humoral antigraft reactions are elusive.

It is puzzling, however, that high levels of preformed alloantibodies before transplantation portend a poor graft outcome in recipients. Likewise, rejections in the presence of circulating anti-donor antibodies carry a worse prognosis than rejections in the absence of such antibodies. It appears that studies of humoral alloreactivity are hampered by the lack of indicating histological markers in biopsy specimens.

We have developed a diagnostic technique that allows the comprehensive assessment of humoral alloreactions in graft biopsies. Our technique takes into account several important aspects as can be seen below.

Endothelial cells within organ grafts form the primary targets for immunological attacks but will remove deposited antibodies and most complement components very rapidly from their surface. Conventional immunohistochemical staining techniques therefore fail to detect transiently bound humoral immune reactants in graft capillaries. Transient deposition of antibodies can be visualized, however, by the assessment of complement fragment C4d, a stable remainder of classical complement activation within capillaries *in vivo,* Assessment of capillary C4d using an indirect immunoperoxidase staining technique can thus reveal otherwise undetectable humoral antigraft reactions in biopsies. Deposition of complement C4d in interstitial capillaries is a unique finding in renal allografts and is not observed in other immunological renal diseases such as glomerulonephritis or vasculitis.

Using this method, we analyzed biopsies from grafts with delayed function (n=93). Capillary C4d was present in half of the biopsies from transplants with DOF and was encountered predominantly in vascular rejections, but also in the majority of grafts that showed preservation injury or combined pathological findings. Importantly, capillary C4d was associated with subsequent early graft loss (18 *vs.* 4 losses; p=0.00[27]).

In a second series, we investigated the capillary deposition of C4d in biopsies derived from 218 cadaveric renal grafts.

Capillary C4d was present in 46% of biopsies from first grafts and 72% of regrafts. Grafts with capillary C4d had a markedly shorter survival than grafts without C4d (50% graft survival: 4 *vs.* 8 years; p=0.0001). Among several risk factors, capillary C4d is the strongest predictor of subsequent graft loss in a multivariate analysis. Furthermore, humoral alloreactivity that is detectable within 6 months after transplantation has a much stronger impact on graft survival than alloreactivity beyond this period.

Using a sensitive cytotluorometric method, we could further demonstrate that capillary C4d is indeed related to the presence of either preformed, or *de* novo formed circulating alloantibodies in recipients.

In summary, humoral alloreactivity, manifested by the capillary deposition of complement C4d in graft biopsies, exerts a strong impact on graft survival when it operates within 6 months after transplantation.

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Post-transplant lymphoproliferative disease

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Epstein-Barr virus associated post-transplant lymphoproliferative disease (PTLD) affects approximately 1% of renal transplant recipients, and allograft involvement is reported in 36-100% of cases (1-4). PTLD is the result of excessive immunosuppression leading to uncontrolled proliferation of Epstein-Barr virus (EBV) transformed B-cells, The clinical presentation varies from a mild febrile syn-