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Influence of surveillance renal allograft biopsy on diagnosis and prognosis of polyomavirus-associated nephropathy

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Influence of surveillance renal allograft biopsy on diagnosis and prognosis of polyomavirus-associated nephropathy.

Background. Polyomavirus-associated nephropathy (PVAN) is an increasingly prevalent cause of allograft dysfunction.

Methods. In 18 histologically proven cases of PVAN managed by reduced immunosuppression, monitoring of serum creatinine, and repeated biopsy, graft outcomes were correlated with clinical and histologic indices. Six months postdiagnosis the status of each graft was classified as poor ($N = 7$) or satisfactory ($N = 11$). Poor transplant status was defined as graft loss, increased severity of PVAN on repeat biopsy, or serum creatinine >3.0 mg/dL. Diagnosis resulted from either surveillance allograft biopsies ($N = 8$) or biopsies performed for increased serum creatinine (nonsurveillance, $N = 10$).

Results. The surveillance biopsy group was more likely than the nonsurveillance group to have satisfactory graft status at 6 months (eight of eight vs. three of ten, $P = 0.004$) and had significantly lower serum creatinine at diagnosis, 3, and 6 months. Histologic scoring for chronic interstitial and tubular injury was lower in diagnostic surveillance biopsies compared to nonsurveillance biopsies ($P = 0.01$). Satisfactory transplant status was also associated with reduced or absent virus on repeat biopsy ($P = 0.01$). Poor transplant status was associated with a higher frequency of recipient^{neg}/donor^{pos} cytomegalovirus (CMV) serology (71% vs. 9%, $P = 0.01$).

Conclusion. Surveillance allograft biopsy provides an important means for earlier detection of PVAN and permits timely alterations to immunosuppression. Early diagnosis is associated with a lesser degree of interstitial fibrosis at diagnosis and lower baseline and subsequent serum creatinine.

Polyomavirus-associated nephropathy (PVAN), caused by the BK virus (BKV) subtype, has emerged over the last 3 years as a postrenal transplant infection with important effects [1–6]. Within this short period of time, it has been reported from transplant centers in the United States and Europe that between 3% and 5% of renal transplant recipients now develop PVAN with rates of graft loss as high as 40% among those affected [6–12]. The disease typically presents as increased serum creatinine and is confirmed by a graft biopsy demonstrating cytopathic change of renal tubular epithelial cells accompanied by an interstitial inflammatory infiltrate of variable severity [6–9, 11]. BKV can be specifically detected within graft tissue by in situ hybridization or immunohistochemistry and in serum by polymerase chain reaction (PCR). The presence of virally loaded cells can also be detected in urine by cytologic or by electron microscopic techniques [6–19]. Treatment has remained a challenge as no prophylactic therapy exists and the single antiviral agent for which some efficacy has been reported (cidofovir) has significant renal toxicity [abstract; Tzuner et al, *Am J Transplant* (Suppl 1):S270, 2000]. While reduction in immunosuppression remains the standard of care, many patients enter a disheartening cycle of alternating PVAN, acute rejection, and progressive graft dysfunction [8]. As proposed by Nickeleit et al [8, 20], a strategy for identification of patients at risk for PVAN and diagnosis of the condition prior to the development of graft injury will be essential for reducing the future impact of this infection and may be associated with complete histologic resolution.

Primary infection with BKV typically occurs in early childhood with an adult seroprevalence rate of 80% [6–9, 21]. The virus remains latent in urothelium and reactivation is often the result of immunosuppression. Following renal transplantation, asymptomatic shedding of virally loaded urothelial "decoy" cells can be detected in the urine in 10% to 30% of recipients [6–9]. With such a large proportion of graft recipients at theoretical risk for PVAN, it is not feasible to reduce immunosuppression or carry out trials of potentially toxic antiviral agents in all individuals with evidence of viral exposure or of viral

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activity within the urothelium. The identification of patients in whom the disease has progressed to the stage of viral activity within renal tubular epithelial cells is central, therefore, to understanding the safety and efficacy of currently available therapeutic interventions for PVAN. The most appropriate means to carry out surveillance for clinically significant BKV activity remains to be determined.

The factors influencing clinical outcomes following diagnosis of PVAN are also poorly understood at present. While earlier studies report high rates of graft loss [1–6], it is likely that these represent predominantly advanced cases. With a growing awareness of PVAN among renal transplant physicians and pathologists as well as the introduction of more sensitive diagnostic modalities, it is likely that the clinical disease spectrum and range of outcomes is broadening [9, 11]. While a number of studies have addressed the utility of different diagnostic modalities to more readily detect PVAN [10–19], none to our knowledge has examined the importance of early histologic disease diagnosis in regards to graft outcome. We report here that the diagnosis of PVAN at a subclinical level by surveillance renal allograft biopsy is associated with significantly improved graft prognosis compared with cases diagnosed by nonsurveillance biopsy during the same time period and managed by similar reductions in immunosuppression. Our results highlight the prognostic importance of early PVAN detection and, with the emergence of this disease as a major cause of graft injury, provide additional supportive evidence for the clinical utility of surveillance renal allograft biopsy.

METHODS

Patient population and identification of PVAN cases

Between September 1996 and December 2001, a total of 672 kidney transplants were performed at Mayo Clinic, Rochester. Surveillance allograft biopsies were performed in all consenting graft recipients at 3 to 4 months and at 12 months posttransplantation. Additional (*nonsurveillance*) biopsies were performed as clinically indicated for unexplained increases in serum creatinine. Biopsies were evaluated by two renal pathologists utilizing standard light microscopy. A diagnosis of PVAN was suspected in biopsies showing focal interstitial mononuclear inflammatory cell infiltrates, dilated tubules with necrotic tubular epithelium, and homogeneous intranuclear inclusion bodies. In situ hybridization for BKV DNA was performed on paraffin-embedded tissue sections from all biopsies for which light microscopic findings were suspicious for PVAN. The tissue sections were deparaffinized and treated with proteinase K, following which a biotin-labeled BKV-specific cDNA probe (Enzo Diagnostics, Inc., Farmingdale, NY, USA) or an appropriate negative control probe were applied. After hybridization, streptavidin-alkaline phosphatase was applied and the hybridization product was visualized by bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (BCIP/NBT) colorimetric reaction. The specificity of the in situ hybridization assay was validated by demonstration of negative staining on tissue samples know to be positive for JC virus (JCV), Epstein-Barr virus (EBV), and cytomegalovirus (CMV). Eighteen patients in whom a renal allograft biopsy demonstrated histologic features diagnostic of PVAN were identified. Five of the renal allograft biopsies that were positive for BKV by in situ hybridization were negative when hybridized with probes for EBV and CMV.

Clinical management and follow-up of PVAN cases

Following the diagnosis of PVAN, stepwise reduction in immunosuppression was carried out. Immunosuppression changes consisted of (*1*) reduction of baseline mycophenolate mofetil dosage (typically 1 g or 750 mg twice daily) by increments of 250 mg twice daily every 2 weeks to a baseline dose of between 500 mg twice daily and 0 mg, and (*2*) reduction in tacrolimus from baseline target trough range (8 to 10 ng/mL or 6 to 10 ng/mL depending on time from transplantation) to a target trough level of between 5 and 7 ng/mL or conversion from tacrolimus to cyclosporine with a target trough level of between 125 and 175 mg/mL. Prednisone dosages were unchanged and were between 5 mg and 10 mg daily. Measurements of serum creatinine were performed weekly following diagnosis for the first 12 weeks, twice monthly for 3 months, then monthly thereafter. Repeated biopsies to ascertain disease status were obtained between 3 and 6 months postdiagnosis in all but five cases, two of whom suffered early graft loss.

Histologic scoring for PVAN severity and chronicity

The severity of BK graft infection by in situ hybridization was graded on a scale of 0 to 5 for all diagnostic and follow-up biopsies. Grading was as follows: 0 = no evidence of disease, 1 = mild disease (nuclear positivity in 10 or fewer tubular cross-sections), 2 = mild-moderate (nuclear positivity in 11 to 20 tubular cross-sections), 3 = moderate (nuclear positivity in 21 to 30 tubular cross-sections), 4 = moderate-severe (nuclear positivity in 31 to 40 tubular cross-sections), and 5 = severe disease (nuclear positivity in greater than 40 tubular cross-sections). The severity of chronic tubular, interstitial, and vascular changes were scored using standard Banff criteria for chronic renal allograft injury based on the degree of interstitial fibrosis, tubular atrophy, arterial fibrous intimal thickening, and hyaline arteriolosclerosis [22].

Data acquisition and statistical analysis

Demographic, clinical, and laboratory data were assimilated in a retrospective fashion on all patients diagnosed with PVAN by review of clinical records. Statisti-

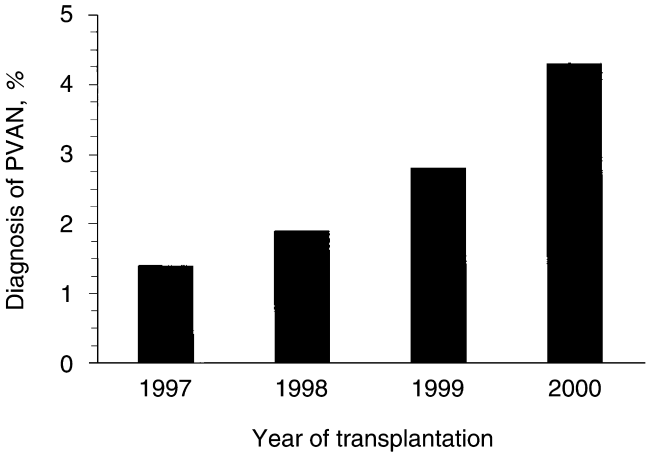

Fig. 1. Diagnosis of polyomavirus-associated nephropathy (PVAN) among renal transplants according to year of transplant. For each year between 1997 and 2000, the percentage of total renal transplant recipients that subsequently received a diagnosis of histologically proven PVAN on or before December 31, 2001 was calculated. A sequential increase in the frequency of PVAN is graphically demonstrated.

Table 1. Characteristics of 18 patients with polyomavirus-associated nephropathy (PVAN)

Age *mean* ± *SD*	56.8 ± 14.4
Male *number* (%)	12 (67)
Cadaveric transplant *number* (%)	5 (28)
Number of MHC mismatches *mean* ± *SD*	2.6 ± 1.9
CMV recipient (−) at time of transplantation *number* (%)	7 (39)
Antibody induction *number* (%)	10 (56)
Immunosuppressive medications prior to PVAN *number* (%)	
Prednisone	18 (100)
Tacrolimus	18 (100)
Cyclosporine	4 (22)
Mycophenolate mofetil	16 (89)
Sirolimus	4 (22)
Acute rejection prior to PVAN diagnosis *number* (%)	5 (28)
Months posttransplant to PVAN diagnosis *mean* ± *SD*	13.8 ± 11
Acute rejection after PVAN diagnosis *number* (%)	4 (22)
Poor transplant status 6 months following PVAN diagnosis[a]	8 (40)

Abbreviations are: MHC, major histocompatibility complex; CMV, cytomegalovirus.

[a]See text for definition of poor transplant status

cal analysis was performed using the SAS software package (SAS Institute, Inc., Cary, NC, USA). The level of statistical significance for all tests was set at P value of <0.05. The Wilcoxon rank sum test was used to compare medians in independent populations and Fisher's exact test for two by two table associations. Results are presented as counts and percentages for qualitative data and mean ± SD for quantitative data.

RESULTS

Characteristics of PVAN among renal transplant recipients

A total of 18 cases of PVAN were diagnosed on the basis of characteristic histopathologic features on renal allograft biopsy and positive in situ hybridization between January 1999 and December 2001. The patients had received their renal transplants between September 1996 and August 2001. Three patients were multiple organ recipients. One had received a combined liver/kidney transplant, another a living donor kidney transplant followed by a cadaveric pancreas transplant, and a third a living donor kidney following a heart transplant. As shown in Figure 1, when the proportion of total renal transplant recipients for each year between 1997 and 2000 who were subsequently diagnosed with PVAN was determined, there was a clear trend toward an increasing occurrence of the disease within our practice. Of the individuals who received a renal transplant in 2000, 4.25% have, to date, developed histologically proven PVAN. Demographic and clinical characteristics for the 18 cases are summarized in Table 1. Of note, the low proportion of cadaveric donor transplants (28%) reflects the predominance of living donation in our practice and, during this time period, the prevalence of PVAN was similar in both cadaveric (2.2%) and living (2.6%) donor allograft recipients. Other pretransplant characteristics of the group, including age, gender, number of major histocompatibility complex (MHC) mismatches, and serologic status for CMV were also comparable with our total cohort of renal transplant recipients. Regarding immunosuppressive therapy, all 18 patients had received tacrolimus and prednisone prior to the diagnosis of PVAN and the majority (89%) had received mycophenolate mofetil. Of the four patients who had received cyclosporine, two had been changed to tacrolimus before developing PVAN. Of the four who had received sirolimus, three had been changed to tacrolimus and one was receiving a combination of sirolimus and tacrolimus. A majority (56%) had received some form of antibody induction at the time of transplantation, a proportion in keeping with the overall employment of induction therapy at our institution during the same time period. The frequency of acute rejection prior to diagnosis of PVAN was 28%.

Four patients (22%) were diagnosed with acute rejection within 6 months following PVAN diagnosis. Three of these had required treatment for acute cellular rejection prior to PVAN diagnosis. Acute rejection following PVAN was diagnosed when BKV was absent by in situ hybridization but significant mononuclear cell infiltrates with tubulitis were present ($N = 2$) or when viral involvement was clearly diminished but mononuclear cell infiltrates with tubulitis were increased ($N = 2$). Three of four patients with acute rejection were treated with steroid bolus therapy, followed by moderate increase in

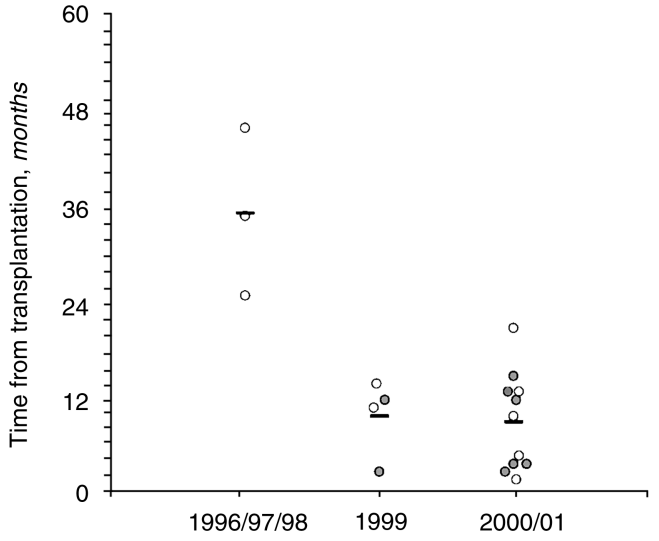

Fig. 2. Time intervals between renal transplantation and diagnosis of histologically proven polyomavirus-associated nephropathy (PVAN) according to year of transplantation. Mean intervals are shown as horizontal bars and individual cases as circles. Open circles denote cases diagnosed by nonsurveillance biopsy; closed circles denote cases diagnosed on surveillance biopsy.

baseline oral immunosuppression and one was treated by increased oral immunosuppression only. To date, two patients have retained stable graft function without evidence of PVAN recurrence and two have suffered graft loss.

The mean interval between transplantation and diagnosis of PVAN was 13.8 months; however, as shown in Figure 2, there has been a clear trend toward more early diagnoses for patients transplanted in 1999 or later compared to those transplanted between 1996 and 1998. For the 11 cases occurring in patients who received a renal transplant in 2000 or 2001, the mean time of diagnosis was 9.3 months posttransplant compared with 10 months for those transplanted in 1999 and 35.3 months prior to 1999. The lack, to date, of later cases among the 1999 cohort suggests that diagnosis within the first year is now characteristic of PVAN.

Effect of PVAN on renal allograft function

Following histologic diagnosis, patients with PVAN were managed by step-wise reductions in immunosuppression and close follow-up of graft function. Repeat allograft biopsy within the first 6 months following diagnosis was carried out in 13 cases. In order to stratify the cases according to the impact of PVAN diagnosis on renal graft function, we divided the patients into those with satisfactory transplant status and those with poor transplant status at 6 months following histologic diagnosis. Poor transplant status was defined as one or more of the following: (*1*) graft loss, (*2*) increased severity of PVAN histologic features on repeat allograft biopsy, or (*3*) serum creatinine greater than 3.0 mg/dL at 6 months following diagnosis. Satisfactory transplant status was defined as (*1*) histologic improvement in PVAN on repeat biopsy and/or (*2*) stable serum creatinine of less than 3.0 mg/dL at 6 months following diagnosis. By these criteria, seven cases (39%) were characterized as having poor and 11 (61%) as having satisfactory transplant status. A comparison of the two groups is presented in Table 2. As shown, there were no significant differences between the groups for age, gender, source of donor organ, MHC mismatches, acute rejection prior to PVAN diagnosis, or exposure to individual oral immunosuppressive agents. Although there was a trend toward later diagnosis in patients with subsequent poor transplant status compared to those with satisfactory status, this did not reach statistical significance. Patients with poor status at 6 months postdiagnosis were significantly more likely to have recipient^{neg}/donor^{pos} CMV serologic status pretransplant (71% vs. 9%, $P = 0.01$), and were less likely to have received antibody induction therapy (17% vs. 82%, $P = 0.01$) or to have improvement in histologic features of PVAN on follow-up biopsy (40% vs. 100%, $P = 0.04$). Strikingly, while 73% of the patients with subsequent satisfactory transplant status had the diagnosis of PVAN made first on a surveillance allograft biopsy, none of the patients with subsequent poor transplant status were diagnosed from a surveillance biopsy ($P = 0.004$). An example of the histologic changes of PVAN from a surveillance renal allograft biopsy is shown in Figure 3.

Diagnosis of PVAN by surveillance renal allograft biopsy is associated with improved graft outcomes and reduced chronic interstitial graft injury

A comparative analysis of baseline characteristics, graft function following the reduction of immunosuppression, and histologic indices of graft injury was carried out for patients in whom PVAN was diagnosed by surveillance and nonsurveillance biopsy (Table 3). Nonsurveillance biopsy diagnosis was, on average, made later following transplantation than surveillance biopsy diagnosis (18.2 $\pm$ 13.8 months vs. 7.6 $\pm$ 4.7 months) but this did not reach statistical significance. The groups also did not differ significantly with regard to donor source, prior acute rejection, use of antibody induction, or exposure to individual oral immunosuppressants. In contrast, however, all surveillance cases were associated with satisfactory transplant status at 6 months postdiagnosis compared with only 30% of the nonsurveillance cases ($P = 0.04$). This observation was reinforced by a comparison of serum creatinine concentrations between both subsets at baseline, 3, and 6 months postdiagnosis (Figure 4). As shown, mean serum creatinine was significantly lower at each time point in the surveillance group. In the nonsurveillance group, there was a trend toward progressive

Table 2. Clinical characteristics of polyomavirus-associated nephropathy (PVAN) cases according to graft function at 6 months postdiagnosis

	Poor transplant status ($N = 7$)[a]	Satisfactory transplant status ($N = 11$)[a]	P value
Months posttransplant to PVAN diagnosis *mean ± SD*	19.9 ± 15.5	9.9 ± 6.9	0.27
Age *mean ± SD*	55.0 ± 11.0	57.3 ± 16.6	0.62
Male *number* (%)	4 (57)	8 (73)	0.63
Cadaveric transplant *number* (%)	3 (43)	2 (18)	0.32
CMV recipient^{neg} at transplantation *number* (%)	5 (71)	2 (18)	0.05
CMV recipient^{neg} donor^{pos} at transplantation *number* (%)	5 (71)	1 (9)	0.01
Number of MHC mismatches *mean ± SD*	2.7 ± 2.2	2.4 ± 1.9	0.68
Available follow-up *months* (mean ± SD)	15.4 ± 7.1	10.5 ± 8.0	0.13
Antibody induction *number* (%)	1 (17)	9 (82)	0.01
Acute rejection prior to PVAN *number* (%)	3/5 (60)	2/10 (20)	0.25
Antibody for rejection prior to PVAN *number* (%)	2/5 (40)	2/10 (20)	0.56
Immunosuppression prior to PVAN *number* (%)			
Prednisone	7 (100)	11 (100)	1.00
Tacrolimus	7 (100)	11 (100)	1.00
Cyclosporine	3 (43)	1 (9)	0.24
Mycophenolate mofetil	6 (86)	10 (91)	1.00
Sirolimus	2 (29)	2 (18)	1.00
PVAN improved on repeat biopsy *number* (%)	2/5 (40)[b]	8/8 (100)[c]	0.04
Diagnosis made on surveillance biopsy	0/7 (0)	8/11 (73)	0.004

Abbreviations are: CMV, cytomegalovirus; MHC, major histocompatibility complex.
[a]Transplant status 6 months following histologic diagnosis of PVAN
[b]No repeat biopsy carried out in two cases
[c]No repeat biopsy carried out in three cases

increase in serum creatinine during the 6 months following PVAN diagnosis with graft loss occurring in two patients (represented graphically as a creatinine value of 7 mg/dL).

The histologic features of PVAN at the time of diagnosis were compared for surveillance and nonsurveillance cases (Table 4). All biopsies were considered adequate by Banff criteria. The extent of viral infection of tubular epithelium was scored using a PVAN severity index (see the **Methods** section). The mean score at diagnosis was not significantly higher for nonsurveillance as compared to surveillance biopsy cases (3.00 vs. 2.14, $P = 0.32$). In contrast, using Banff criteria, indices of chronic interstitial and tubular injury were significantly higher at the time of diagnosis for nonsurveillance as compared to surveillance cases (interstitial chronicity, 2.20 ± 0.92 vs. 1.00 ± 0.63, $P = 0.02$; tubular chronicity, 2.40 ± 0.84 vs. 1.3 ± 0.52, $P = 0.02$). For six of the nonsurveillance cases and seven of the surveillance biopsy cases a follow-up biopsy was carried out between 3 and 6 months following PVAN diagnosis. Strikingly, a significant reduction in the PVAN severity score between diagnosis and follow-up biopsy was observed for surveillance biopsy cases (2.14 ± 1.94 vs. 0.75 ± 0.96, $P < 0.05$), whereas PVAN severity was essentially unchanged for the nonsurveillance biopsy group (3.00 ± 1.63 vs. 3.00 ± 2.00). Four cases were encountered for which in situ hybridization for BKV was negative on the repeat biopsy. All of these cases occurred within the group diagnosed by surveillance biopsy. There were also four cases, all within the nonsurveillance biopsy group, for which PVAN severity was increased on repeat biopsy. Although the numbers of cases of histologically confirmed "viral clearance" and "viral progression" did not allow for a detailed comparison, the observation emphasizes the potential for elimination of BKV from the graft in the context of early diagnosis as well as the importance of follow-up biopsy in determining the success of interventional strategies [20]. Additional histologic analyses that were carried out on baseline and follow-up biopsies included

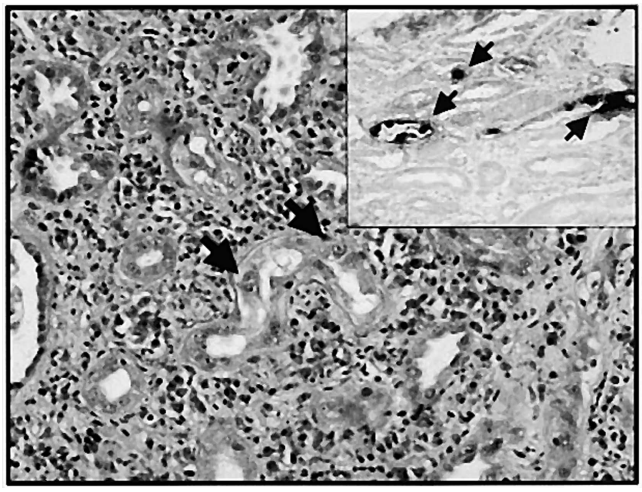

Fig. 3. An example of histologic injury due to polyomavirus-associated nephropathy (PVAN) on a surveillance renal allograft biopsy. A renal allograft surveillance biopsy at 1-year posttransplant demonstrates dense interstitial mononuclear infiltration and cytopathic changes of multiple tubular epithelial cells suggestive of PVAN (large arrows). In situ hybridization for BKV DNA confirms the diagnosis with nuclear positivity of individual cells from multiple tubular cross-sections (small arrows). The patient was a 43-year-old woman who had received a living unrelated donor renal transplant. At the time of biopsy, serum creatinine was stable at 1.5 mg/dL.

Table 3. Clinical characteristics of polyomavirus-associated nephropathy (PVAN) cases according to diagnostic modality

	Surveillance biopsy ($N = 8$)	Nonsurveillance biopsy ($N = 10$)	P value
Months posttransplant to PVAN diagnosis *mean* ± *SD*	7.6 ± 4.7	18.2 ± 13.8	0.08
Cadaveric transplant *number* (%)	1 (12.5)	4 (40)	0.31
Acute rejection prior to PVAN diagnosis *number* (%)	2 (25)	3 (30)	1.00
Antibody induction *number* (%)	6 (75)	5 (50)	0.37
Immunosuppression prior to PVAN *number* (%)			
Prednisone	8 (100)	10 (100)	1.00
Tacrolimus	8 (100)	10 (100)	1.00
Cyclosporine	1 (12.5)	3 (30)	0.58
Mycophenolate mofetil	7 (87.5)	9 (90)	1.00
Sirolimus	1 (12.5)	2 (20)	1.00
Poor transplant status 6 months following PVAN diagnosis *number* (%)	0/8 (0)	7/10 (70)	0.04

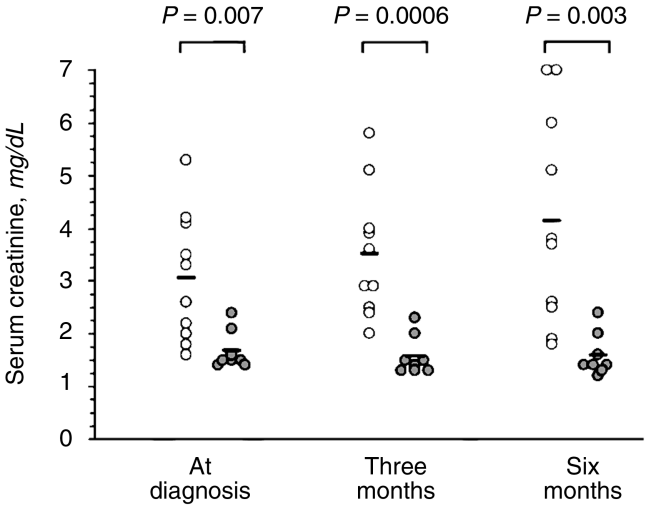

Fig. 4. Serum creatinine values at baseline and during follow-up are significantly lower for cases diagnosed by surveillance biopsy compared to nonsurveillance biopsy. Serum creatinine at the time of histologic diagnosis of polyomavirus-associated nephropathy (PVAN) and at 3 and 6 months postdiagnosis are shown for cases diagnosed by nonsurveillance biopsy (open circles) and by surveillance biopsy (closed circles). Cases in which graft loss occurred are represented graphically as having serum creatinine of 7.0 mg/dL at 6 months. Mean values for the groups at each time point are represented as horizontal bars. Significantly lower mean serum creatinine values were observed for protocol compared with nonprotocol cases at diagnosis as well as at the two follow-up time points.

Banff scoring for acute and chronic glomerular and vascular injury. For these analyses no significant differences were observed between surveillance and nonsurveillance biopsies (data not shown). Despite stable creatinine values and frequent apparent elimination of intragraft BKV during the follow-up period, there was a trend toward increased tubular chronicity on follow-up biopsy for cases diagnosed by surveillance biopsy.

DISCUSSION

The most significant finding of this report is the superior prognosis for PVAN in renal transplant recipients in whom the diagnosis was made on a surveillance allograft biopsy. Importantly, the cases identified from surveillance and nonsurveillance biopsies were diagnosed during the same recent time period, received similar immunosuppressive management both before and after the diagnosis of PVAN, and did not have significantly different initial viral burden based on in situ hybridization. In fact, the two groups primarily differed at the time of diagnosis with regard to serum creatinine and degree of tubulointerstitial scarring. Although these findings suggest that histologic diagnosis of PVAN at an early stage can prevent subsequent irreversible graft injury, we cannot entirely rule out the possibility that the two groups differ in other respects such as initial viral burden, viral strain, or genetic predisposition to aggressive disease. The fact that the prevalence of PVAN in our practice over the past 3 years closely parallels reported rates from centers in which all cases are associated with increased serum creatinine [6, 9–12] suggests, however, that the cases diagnosed by surveillance biopsy in this study would eventually have manifested graft dysfunction as a result of PVAN. We interpret these observations, therefore, as a strong indication that the diagnosis of PVAN prior to the onset of clinical graft dysfunction is both feasible and desirable.

Our results can also be viewed in the context of a growing appreciation that clinically manifest graft injury may be preceded by detectable subclinical disease and that intervention during this phase may beneficial [23, 24]. Hirsch [9] and Nickeleit et al [3, 8, 20] have proposed a model of PVAN pathogenesis in which asymptomatic urinary viral shedding is followed by early graft invasion with detectable viremia and, subsequently, by clinical graft dysfunction with overt histologic disease. Consistent with this model there is evidence for a predictable histologic sequence in PVAN with the earliest stage consisting of cytopathic changes within clusters of medullary tubular epithelial cells [9, 11, 25]. Progression of the disease process is characterized by viral invasion of all tubular sections with associated tubular degeneration and

Table 4. Histologic staging at diagnostic and 3-month follow-up biopsy in patients with polyomavirus-associated nephropathy (PVAN)

	Diagnostic biopsy			Follow-up biopsy		
	Nonsurveillance ($N = 10$)	Surveillance ($N = 8$)	P value[a]	Nonsurveillance ($N = 6$)	Surveillance ($N = 7$)	P value[a]
PVAN severity index	3.00 ± 1.63	2.14 ± 1.95	0.34	3.00 ± 2.00	0.75 ± 0.96	0.08
Interstitial chronicity index	2.20 ± 0.92	1.00 ± 0.63	0.01	2.30 ± 0.80	1.30 ± 0.96	0.09
Tubular chronicity index	2.4 ± 0.84	1.30 ± 0.52	0.01	2.20 ± 0.80	2.00 ± 0.82	0.75

[a]Two-sample test, equal variance

inflammatory infiltrates. A late stage, in which tubular atrophy and interstitial fibrosis are predominant and viral cytopathic changes infrequent, may be indistinguishable from chronic allograft nephropathy. Drachenberg et al [11], in an extensive series of biopsy-proven cases, provide clear examples of this histologic progression. Furthermore, in a series of cynomolgous monkeys immunosuppressed for experimental transplantation, Van Gorder et al [25] have described similar histologic stages for a simian PVAN that closely resembles the human disease. Interestingly, in these monkeys, the timing of histologic onset in renal allografts appeared to occur predictably between 3 and 8 weeks after transplantation. Our observation that biopsies carried out after a rise in serum creatinine contain significantly more chronic tubulointerstitial scarring is in keeping with this model of progression and we propose that a substantial proportion of PVAN cases can be detected prior to the onset of graft dysfunction by appropriately timed surveillance biopsies.

The invasive nature of an allograft biopsy raises the important question of whether a noninvasive assay can be employed to provide similar early diagnosis of PVAN. This has been the focus of a number of recent reports in which the correlation between histologic PVAN and blood or urine testing for BKV has been examined [10-12, 16-19] [abstract; Vats et al, *Transplantation* (Suppl 69):S136, 2000]. The presence of decoy cells in urine appears to be an invariable finding at the time of histologic PVAN diagnosis, and has an excellent negative predictive value [8, 9, 11]. Nickeleit et al [8] have suggested that urine cytology can be used as a first-line screening test to identify individuals requiring further monitoring. PCR testing of urine has been examined prospectively by several investigators [17, 19]. Ding et al [19] have reported that sequential quantitative PCR of urine may identify a "threshold viral load" for predicting the occurrence of PVAN. Two recent studies have demonstrated the presence of BKV in blood samples by PCR at the time of histological diagnosis of PVAN [10, 16]. These reports also showed clearly that detectable BKV in blood can predate the development of histologic PVAN and that reduction in immunosuppression can be rapidly followed by disappearance of virus from the blood. More recently, Hirsch et al [12] have demonstrated prospectively that BK viremia can be detected in 13% of renal allograft recipients during the first 2 years following transplantation. While these observations support the use of surveillance by blood PCR as a sensitive means to identify recipients at heightened risk for PVAN, it is not yet clear whether reduction in immunosuppression is justified at the time of a first positive blood test if a biopsy proves negative. Clearly, additional prospective studies involving large patient numbers will be needed to develop robust screening strategies to identify recipients in the early stages of graft involvement while avoiding immunosuppression reduction or antiviral use in those with nonpathogenic viruria or viremia.

The recent emergence of PVAN is likely a reflection of the use of newer, more potent immunosuppressive regimens [2–9]. Whether one or more of these immunosuppressants is specifically responsible for fostering BK viral invasion of renal tubular epithelium remains less clear [9]. As with the majority of reported cases, our patients have predominantly received a tacrolimus/mycophenolate–based immunosuppressive regimen. However, in common with other studies, we cannot formally ascribe increased risk to one agent or combination. The issue of disease prognosis under different immunosuppressive regimens also remains unexplored. Interestingly, we find that the use of antibody induction was associated with significantly less risk of poor transplant status 6 months following PVAN diagnosis. This is in contrast to the reported poor prognosis of PVAN following the use of antibody therapy for acute rejection [3, 5]. As prior acute rejection has been commonly reported in clinically severe PVAN, it is possible that induction therapy, by reducing early acute rejection, is associated with less graft injury at the time of viral invasion. It is to be hoped that for some emerging immunosuppressive agents the risk of BKV reactivation will be reduced.

The interaction between CMV infection and other causes of graft injury continues to be a significant consideration in organ transplant recipients [26–30]. It is of interest, therefore, that our analysis suggests an association between the recipient^{neg}/donor^{pos} serologic profile for CMV and poor graft outcome following PVAN diagnosis. Nonetheless, in a recent report by Hirsch et al [12], no temporal association could be demonstrated between

BKV replication and CMV antigenemia in prospectively screened renal transplant recipients. Further prospective studies involving large groups of patients will be required to determine whether the clinical course of PVAN is influenced by primary CMV infection, CMV reactivation, or by other opportunistic infections.

A number of important questions remain regarding the long-term function of grafts affected by PVAN. The 6-month follow-up period that we have focused upon for this analysis, while relatively short, is in keeping with the reported potential for PVAN to progress rapidly toward advanced graft dysfunction or graft loss [1–6]. In addition, we find, in patients diagnosed early by surveillance biopsy, that repeated biopsy within 6 months of altering immunosuppression can demonstrate significantly diminished or even absent viral involvement of tubular epithelium and improvement in short-term allograft survival. In our cohort of patients, reduced immunosuppression following PVAN diagnosis has not been associated with an unacceptable rate of acute rejection (22%). It remains to be seen, however, whether these patients will suffer higher rates of late acute rejection or chronic allograft nephropathy compared to those in whom reduced immunosuppression has not been necessary. In addition, it is not currently known whether relapse of PVAN or progression of interstitial fibrosis following elimination of the virus will be encountered during prolonged follow-up. For patients in whom graft loss occurs as a result of PVAN, the risk of recurrence in a subsequent allograft is also unknown. Short-term success has been reported for retransplantation [31] but longer clinical experiences with PVAN will be necessary before this, and other, important questions are clearly resolved.

CONCLUSION

Our results point to a number of parameters that are associated with poor graft outcome in renal transplant recipients with PVAN. The most important of these is a delay in diagnosis until clinically apparent graft dysfunction and histologic evidence of chronic interstitial injury have occurred. We find that surveillance protocol biopsies allow for detection of invasive BK viral disease at a subclinical stage and, importantly, we show that this early diagnosis is accompanied by reduced risk of subsequent disease progression. We believe that the widespread availability of noninvasive tests for BK viral activity, particularly PCR screening of blood, is likely to provide an additional opportunity for early diagnosis and management of PVAN but that the predictive value of these tests must continue to be validated in the context of histologically confirmed disease.

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