

PROSPECTIVE STUDY OF POLYOMAVIRUS TYPE BK REPLICATION AND NEPHROPATHY IN RENAL-TRANSPLANT RECIPIENTS

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ABSTRACT

Background Nephropathy associated with the polyomavirus type BK (BKV) nephropathy has emerged as a cause of allograft failure linked to immunosuppressive regimens containing tacrolimus or mycophenolate mofetil. The presence of viral inclusions, known as “decoy cells,” in urine and the presence of BKV DNA in plasma have been proposed as markers for the replication of BKV and associated nephropathy, but data from prospective studies have been lacking.

Methods In a prospective, single-center study, we followed 78 renal-transplant recipients who were receiving immunosuppressive therapy that included tacrolimus (37 patients) or mycophenolate mofetil (41 patients). Urine was tested for the presence of decoy cells at routine visits. BKV DNA was measured 3, 6, and 12 months after transplantation and whenever decoy cells were detected. The viral load in plasma was quantified with the use of a real-time polymerase-chain-reaction method. Renal biopsy was performed if allograft function deteriorated.

Results Twenty-three patients had decoy-cell shedding a median of 16 weeks after transplantation (range, 2 to 69), 10 patients had BKV viremia at a median of 23 weeks (range, 4 to 73), and 5 had BKV nephropathy at a median of 28 weeks (range, 8 to 86). Kaplan–Meier estimates of the probability of decoy-cell shedding, viremia, and nephropathy were 30 percent (95 percent confidence interval, 20 to 40 percent), 13 percent (95 percent confidence interval, 5 to 21 percent), and 8 percent (95 percent confidence interval, 1 to 15 percent), respectively. Antirejection treatment, particularly with corticosteroids, was associated with BKV replication and nephropathy. The viral load in plasma was higher in patients with BKV nephropathy than in those without nephropathy ($P < 0.001$ by the Mann–Whitney test). BKV antibodies were detected in 77 percent of the 78 patients before transplantation, including 4 of 5 with BKV nephropathy.

Conclusions BKV nephropathy in renal-transplant recipients represents a secondary infection associated with rejection and its treatment in most cases and could be monitored by measuring the viral load in plasma. (N Engl J Med 2002;347:488-96.)

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SINCE it was first reported in 1995, nephropathy associated with the polyomavirus type BK (BKV) has emerged as an important cause of allograft failure in renal-transplant recipients.¹⁻³ BKV is closely related to another human polyomavirus, JC virus (JCV), which causes progressive multifocal leukoencephalopathy in immunocompromised patients.⁴ Infection with either polyomavirus is widespread, as indicated by seroprevalence rates of up to 90 percent worldwide.⁵ The risk factors for BKV nephropathy in renal-transplant recipients are not known, but most patients with BKV nephropathy have received newer immunosuppressive drugs such as tacrolimus or mycophenolate mofetil.⁶⁻¹³ Because BKV persists in the kidney, the transplantation of organs from seropositive donors into seronegative recipients may lead to BKV nephropathy.¹⁴ According to retrospective studies, BKV nephropathy develops in 1 to 5 percent of renal-transplant recipients, with loss of allograft function occurring in about half the cases.^{2,3} BKV-specific antiviral therapy is not available, but in some cases, BKV replication may be controlled by reducing the level of maintenance immunosuppression,^{9,15} though this change may result in an increased risk of subsequent rejection.¹³

Progression to BKV nephropathy occurs without clinical signs or symptoms, except for increasing serum creatinine concentrations over a period of weeks. Diagnosis of BKV nephropathy is based on the histopathological demonstration of viral alterations that are distinct from signs of rejection in specimens from allograft biopsies.^{2,16,17} Cells in the urine that have viral inclusions, known as “decoy cells,” are a sign of BKV replication in the renourinary tract but are not a specific marker of BKV nephropathy.^{6,7} BKV replication in the allograft has been correlated with the detection of BKV DNA in plasma by polymerase-chain-reaction (PCR) assay.^{13,15} BKV DNA may serve as a quantifiable surrogate marker of the course of the infection.^{18,19} To determine the association between BKV replication

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and nephropathy, we conducted a prospective, single-center study of renal-transplant recipients who were receiving immunosuppressive therapy that included tacrolimus or mycophenolate mofetil.

METHODS

Patients

Between January 1999 and August 2000, 117 patients received kidney transplants and post-transplantation care at University Hospitals in Basel, Switzerland. Of the 117 patients, 95 were treated with an immunosuppressive regimen consisting of tacrolimus, azathioprine, and prednisone or mycophenolate mofetil, cyclosporine, and prednisone. Eight patients were excluded because of a nonfunctioning allograft or nephrectomy within the first two months, 1 patient died from sepsis, and 8 patients declined participation or were not identified by their physicians as eligible for the study, leaving a total of 78 patients available for enrollment in the study.

Thirteen of the 78 patients were considered to be at high risk for rejection because of a value for panel-reactive antibodies that exceeded 25 percent, B-cell reactivity, or loss of a transplant within the previous three years due to rejection. These patients received induction treatment with antilymphocyte preparations (antithymocyte globulin in five patients and anti-interleukin-2 receptor preparations in eight) in addition to cyclosporine, mycophenolate mofetil, and prednisone (Table 1).

Transplant recipients with presumed graft rejection were treated with intravenous corticosteroids until the diagnosis was confirmed by histologic examination of a kidney-biopsy specimen, usually within one to three days. In 20 patients, biopsy of the allograft revealed acute vascular rejection or acute interstitial rejection that was unresponsive to previous corticosteroid treatment and was treated with antilymphocyte globulin. In 30 patients, interstitial rejection was diagnosed and treated with intravenous methylprednisolone (500 mg daily for three to five days). Borderline rejection, according to the Banff classification, was diagnosed and treated if there was immunohistochemical evidence of inflammatory infiltrates and major histocompatibility class II (HLA-DR) expression in renal tubular epithelial cells.^{16,20}

Written informed consent was obtained from all 78 enrolled patients. The study was performed in accordance with the guidelines of the Basel Ethics Committee.

Outcomes

The primary outcomes were the detection of decoy cells in urine (indicating BKV replication), the detection of BKV DNA in plasma (indicating viremia), and the detection of BKV nephropathy in an allograft-biopsy specimen. Urine samples were obtained during routine monthly outpatient visits for the first six months after transplantation, as well as whenever patients were hospitalized, allograft function declined, or biopsy of the allograft was performed. When decoy cells were detected, nested PCR assay was used to measure BKV DNA in plasma. In addition, BKV DNA was measured 3, 6, and 12 months after transplantation. Secondary outcomes were the serum creatinine concentration on day 6 and at the end of the study, in June 2001, and cytomegalovirus (CMV) replication, defined by a positive test for pp65 antigen.

Virologic Studies

EDTA-anticoagulated plasma samples were analyzed with the use of BKV-specific and JCV-specific nested, qualitative PCR assays, as described previously.¹⁵ JCV was not detected in plasma samples from any of the 78 patients studied. BKV viral load was determined in all samples that were positive for BKV according to the BKV-specific nested PCR method. Quantification was performed with the use of a BKV-specific, real-time PCR method (TaqMan ABI Prism 7700,

Applied Biosystems), as described elsewhere¹⁸; this method did not detect JCV genomes or plasmids bearing the cloned homologous sequence of JCV large T antigen. Tests of plasma samples from 33 healthy blood donors had negative results. BKV-specific antibodies were measured by hemagglutination inhibition in serum samples obtained before transplantation from 77 of the 78 patients. A titer of less than 10 was considered to be a negative result, and a titer of 10 or more a positive result.^{5,21} CMV infection was diagnosed with the use of monoclonal antibodies against the pp65 antigen on cytopsin preparations of 500,000 buffy-coat cells. For one patient with BKV nephropathy and CMV antigenemia, the CMV viral load in whole blood was determined with the use of a kit (CMV AmpliCor Monitor, Roche).

Cytologic and Histologic Studies

Urinary cytologic smears stained by the Papanicolaou method were evaluated for the presence or absence of cells with intranuclear viral inclusions (decoy cells); if present, decoy cells were expressed as the number per 10 high-power fields.⁷ Allograft biopsies were performed if the serum creatinine concentration increased by more than 25 percent from the base-line value and if other nonrenal causes were ruled out; biopsy specimens were stained for polyomavirus-specific antigens.⁷ Study personnel involved in generating the data from cytologic, histopathological, and virologic studies were unaware of other laboratory and clinical data on the patients.

Statistical Analysis

The data were analyzed with the use of SPSS software (version 10.0). Pearson's chi-square test, Fisher's exact test, and the Mann-Whitney U test were used to analyze the data, as appropriate. Kaplan-Meier analysis was used to estimate the probability of the study end points. A P value of less than 0.05 (two-sided test) was considered to indicate statistical significance. Factors associated with borderline significance ($P < 0.10$) in the univariate analysis were entered into a Cox proportional-hazards model in a forward, stepwise fashion in order to analyze their effects on the outcomes of decoy-cell shedding, BKV viremia, and BKV nephropathy.

RESULTS

Seventy-eight renal-transplant recipients (37 who were receiving tacrolimus, azathioprine, and prednisone, and 41 who were receiving cyclosporine, mycophenolate mofetil, and prednisone) were prospectively followed for a median of 85 weeks (range, 43 to 130). The characteristics of the patients are shown in Table 1. Shedding of decoy cells in urine was detected in 23 of the 78 patients 2 to 69 weeks after transplantation (median, 16). BKV viremia was present in 10 patients 4 to 73 weeks after transplantation (median, 23). BKV nephropathy was diagnosed in allograft-biopsy specimens from five patients 8 to 86 weeks after transplantation (median, 28). Fifty-five of the 78 patients (71 percent) had no evidence of decoy cells, viremia, or nephropathy. The three BKV outcomes were not associated with either regimen of maintenance immunosuppressive therapy (Table 2). Kaplan-Meier analysis (Fig. 1) showed that the probability of BKV nephropathy was 8 percent (95 percent confidence interval, 1 to 15 percent), the probability of BKV viremia was 13 percent (95 percent confidence interval, 5 to 21 percent), and the probability of decoy-cell shedding was 30 percent (95 percent confidence interval, 20

TABLE 1. CHARACTERISTICS OF 78 RENAL-TRANSPLANT RECIPIENTS.

CHARACTERISTIC	VALUE
Female sex — no. (%)	32 (41)
Age — yr	
Median	51
Range	19–70
Cadaveric donor — no. (%)	40 (51)
HLA mismatches — no.	
Mean \pm SD	3.9 \pm 1.4
Median	4
Range	0–6
Induction therapy with antilymphocyte preparations — no. (%)*	13 (17)
Maintenance regimen — no. (%)	
Tacrolimus, azathioprine, prednisone	37 (47)
Cyclosporine, mycophenolate mofetil, prednisone	41 (53)
Serum creatinine — mg/dl†	
Day 6	
Median	1.9
Range	0.6–13.3
End of study	
Median	1.6
Range	0.8–4.2
Follow-up after transplantation — wk	
Median	85
Range	43–130
Antirejection treatment — no. (%)	
Antilymphocyte globulin	20 (26)
Methylprednisolone	30 (38)
Cytomegalovirus infection — no. (%)‡	27 (35)

*Antilymphocyte globulin was administered in five patients and anti-interleukin-2-receptor globulin in eight.

†To convert the values for creatinine to micromoles per liter, multiply by 88.4.

‡Cytomegalovirus infection was defined by the detection of the pp65 antigen in peripheral-blood leukocytes.

to 40 percent). At the end of the study, 75 of the 78 patients (96 percent) had functioning allografts; there was no graft loss due to BKV nephropathy.

Patients with decoy-cell shedding, those with BKV viremia, and those with nephropathy were more likely to have received antirejection treatment with antilymphocyte preparations or methylprednisolone — particularly a higher number of corticosteroid pulses ($P < 0.05$ by the Mann-Whitney U test) — than were patients without decoy-cell shedding (Table 2). In contrast, induction immunosuppressive therapy with the use of antilymphocyte preparations was not significantly associated with decoy-cell shedding or nephropathy (Table 2). There was a trend toward a higher number of HLA mismatches between donor and recipient in patients with BKV viremia and those with nephropathy than in patients without decoy-cell shedding — those who did not have signs of BKV replication (Table 2). We could not identify an association with other variables such as allograft type (cadaveric

or living donor), duration of cold ischemia, or use of high-level immunophilins (Table 2).

When factors of borderline significance in the univariate analysis ($P < 0.1$) were included in multivariate logistic-regression models, only the number of corticosteroid pulses remained significantly associated with BKV replication and nephropathy ($P = 0.01$; relative risk, 1.21 [95 percent confidence interval, 1.08 to 1.36]; and $P = 0.02$; relative risk, 1.38 [95 percent confidence interval, 1.04 to 1.68], respectively). The number of corticosteroid pulses and the number of HLA mismatches remained significantly associated with BKV viremia ($P = 0.01$; relative risk, 1.28 [95 percent confidence interval, 1.06 to 1.56]; and $P = 0.04$; relative risk, 1.78 [95 percent confidence interval, 1.03 to 3.07], respectively).

CMV antigenemia was significantly associated with the use of antilymphocyte preparations as antirejection treatment but not with the use of intravenous corticosteroids ($P = 0.03$ and $P = 0.73$, respectively, by Pearson's chi-square test), a difference that was not attributable to prophylaxis with ganciclovir. Although some patients had both CMV and BKV replication, the two infections were not significantly associated (Table 2). To determine whether a reactivation of BKV and CMV replication was more likely in patients who were seropositive for both viruses, we compared the time at which decoy-cell shedding was detected with the time at which pp65 antigen was detected. Some patients had concurrent infections, but overall, there was no correlation ($r^2 < 0.01$) (Fig. 2). BKV-specific antibodies were found in serum samples obtained before transplantation from 59 patients (77 percent); the median titer was 80 (range, 10 to 1280). Of the 23 patients with decoy-cell shedding, 18 were seropositive and 5 were seronegative before transplantation, suggesting the possibility of primary infection in the 5 patients who were initially seronegative. Three of these five patients had BKV viremia, yet nephropathy developed in only one. Of the five patients with BKV nephropathy, four were seropositive before transplantation.

We investigated the usefulness of decoy-cell shedding and BKV viremia as diagnostic markers for BKV nephropathy. The sensitivity of decoy-cell shedding for the diagnosis of BKV nephropathy was 100 percent, the specificity was 71 percent, the positive predictive value was 29 percent, and the negative predictive value was 100 percent when matched allograft-biopsy samples were used as the diagnostic standard. BKV viremia had a diagnostic sensitivity of 100 percent, a specificity of 88 percent, a positive predictive value of 50 percent, and a negative predictive value of 100 percent. The mean viral load in plasma was significantly higher in patients with biopsy-proven BKV nephropathy than in patients without histologic evidence of nephropathy (28,000 copies per milliliter vs. 2000

TABLE 2. DECOY-CELL SHEDDING, BKV DNA, AND BKV NEPHROPATHY.*

VARIABLE	NO DECOY CELLS (N=55)	DECOY CELLS (N=23)	BKV DNA (N=10)	BKV NEPHROPATHY (N=5)	P VALUE†	NO DECOY CELLS VS. DECOY CELLS	NO DECOY CELLS VS. VIREMIA	NO DECOY CELLS VS. NEPHROPATHY
BKV seroprevalence before transplantation — no. (%)	41 (76)‡	18 (78)	7 (70)	4 (80)	0.69	0.83	0.69	1.00
Cadaveric donor	29 (53)	11 (48)	4 (40)	2 (40)	0.46	0.69	0.46	0.67
Duration of cold ischemia — min					0.27	0.48	0.27	0.21
Median	628	74	66	54				
Range	1–2640	34–3560	34–835	34–835				
Sex mismatch between donor and recipient — no. (%)	28 (51)	14 (61)	7 (70)	4 (80)	0.41	0.40	0.41	0.39
No. of HLA mismatches between donor and recipient					0.07	0.40	0.07	0.09
Mean ±SD	3.8±1.4	4.1±1.3	4.7±1.1	4.8±1.1				
Median	4	4	5	5				
Range	0–6	2–6	3–6	3–6				
Induction therapy with antilymphocyte preparations — no. (%)	10 (18)	3 (13)	1 (10)	1 (20)	0.53	0.58	0.53	1.00
Maintenance regimen containing tacrolimus — no. (%)	26 (47)	11 (48)	6 (60)	3 (60)	0.46	0.96	0.46	0.67
High-level tacrolimus§	13 (24)	6 (26)	4 (40)	2 (40)	0.19	0.82	0.19	0.59
Maintenance regimen containing cyclosporine and mycophenolate mofetil — no. (%)	29 (53)	12 (52)	4 (40)	2 (40)	0.46	0.96	0.46	0.67
High-level cyclosporine¶	5 (9)	2 (9)	0	0	0.32	0.96	0.32	1.00
Antirejection treatment with ALG — no. (%)	11 (20)	9 (39)	6 (60)	3 (60)	0.008	0.07	0.008	0.08
Antirejection treatment with corticosteroid pulses — no. (%)	30 (55)	20 (87)	10 (100)	5 (100)	0.007	0.007	0.007	0.07
No. of corticosteroid pulses					0.004	0.005	0.004	0.007
All patients	2.6±2.9	5.2±3.7	6.6±3.4	7.0±3.1				
Mean ±SD	3	3	7	7				
Median	0–10	0–12	2–11	3–10	0.03	0.05	0.03	0.01
Range								
Patients not receiving ALG								
Mean ±SD	1.9±2.3	4.1±3.8	6.3±4.0	8.5±2.1				
Median	0	3	7	9				
Range	0–9	0–12	2–10	7–10				
CMV antigenemia — no. (%)	17 (31)	10 (43)	4 (40)	2 (40)	0.29	0.29	0.29	0.65
Hydronephrosis — no. (%)	16 (29)	5 (22)	3 (30)	1 (20)	0.95	0.50	0.95	1.00

*BKV denotes polyomavirus BK, ALG antilymphocyte globulin, and CMV cytomegalovirus.

†P values were calculated with the use of Pearson's chi-square test or Fisher's exact test, as appropriate; the Mann-Whitney U test was used for comparisons of the duration of cold ischemia, the number of HLA mismatches, and the number of corticosteroid pulses.

‡A pretransplantation serum sample was unavailable for one patient.

§High-level tacrolimus was defined by a trough blood level that exceeded 15 ng per milliliter for more than seven days at any time, excluding the first four weeks after transplantation.

¶High-level cyclosporine was defined by a trough blood level that exceeded 400 ng per milliliter for more than seven days at any time, excluding the first four weeks after transplantation.

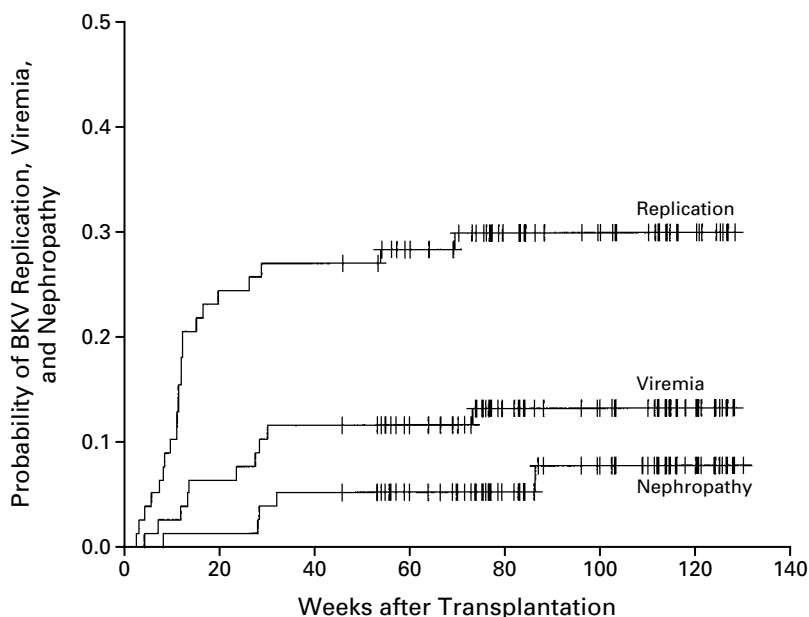


Figure 1. Kaplan–Meier Estimates of BK Virus (BKV) Replication, Viremia, and Nephropathy in 78 Renal-Transplant Recipients. Viral replication was defined by the presence of decoy cells in urine. Viremia was defined by the detection of BKV DNA in plasma by the polymerase-chain-reaction assay. BKV nephropathy was diagnosed on the basis of histologic findings in allograft-biopsy specimens.

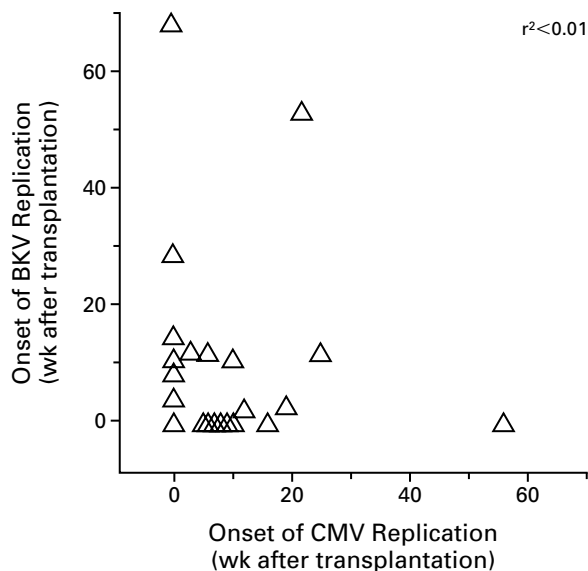


Figure 2. Onset of BK Virus (BKV) and Cytomegalovirus (CMV) Replication in Patients Who Were Seropositive for Both Viruses. BKV replication was defined by the presence of decoy cells in urine, and CMV replication by the presence of pp65 antigen in peripheral-blood leukocytes.

copies per milliliter; $P < 0.001$ by the Mann–Whitney U test). The viral load increased to 7700 copies per milliliter or more in all the patients in whom BKV nephropathy developed; in three of five patients, the viral load rose to 10,000,000 copies per milliliter. In contrast, the viral load remained low in patients without BKV nephropathy.

Four of the five patients with BKV nephropathy had evidence of concurrent acute interstitial rejection in samples from allograft biopsies. In these patients, a modification of the maintenance immunosuppressive regimen was combined with antirejection treatment (Fig. 3).

DISCUSSION

BKV-mediated allograft dysfunction has been retrospectively identified in 1 to 5 percent of renal-transplant recipients, but the incidence of BKV nephropathy, risk factors for it, and appropriate diagnostic procedures have not been completely elucidated.^{2,3} In our prospective, single-center study involving 78 renal-allograft recipients, all of whom were being treated with immunosuppressive regimens containing either tacrolimus or mycophenolate mofetil, the estimated probability of BKV replication (as indicated by decoy-

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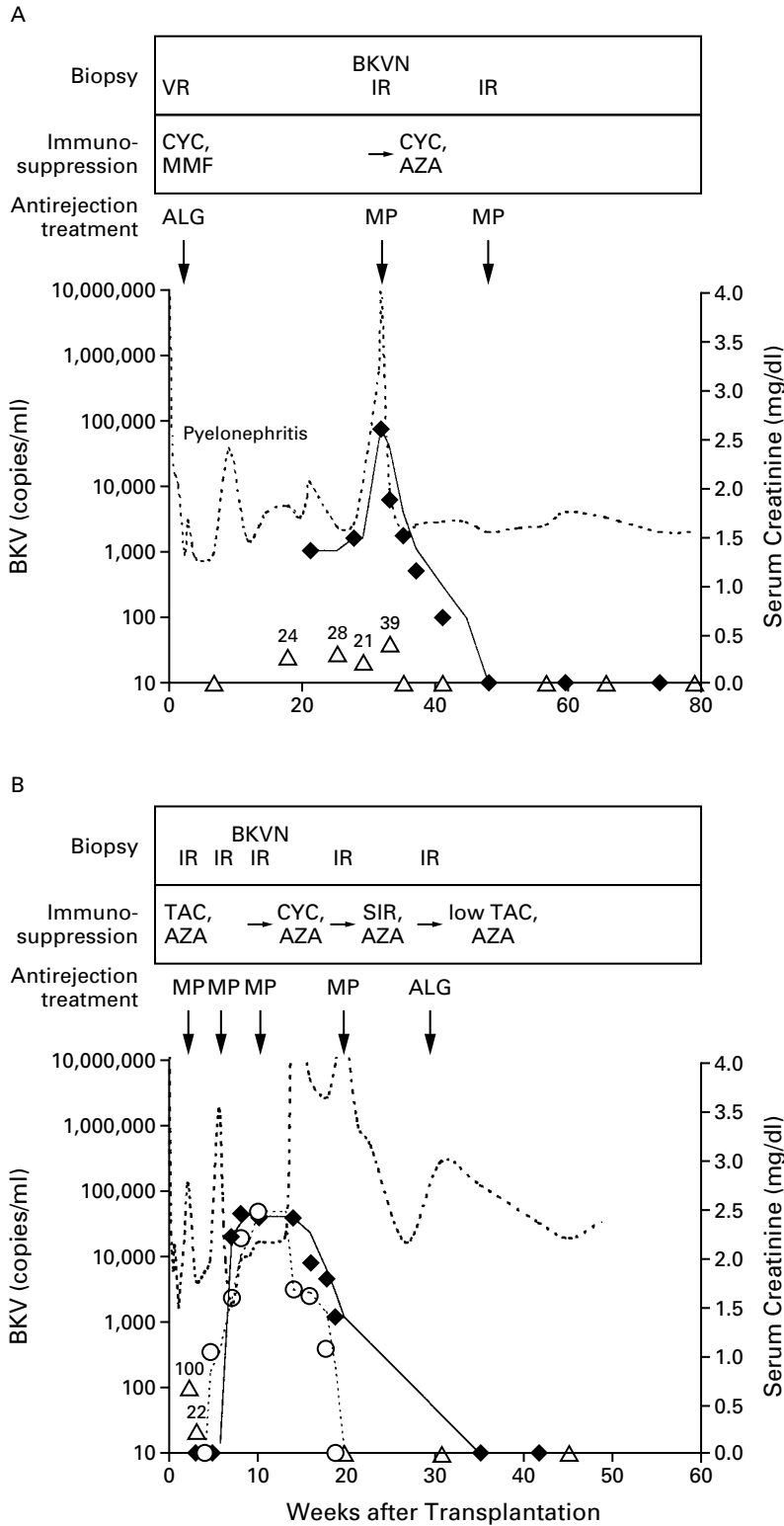
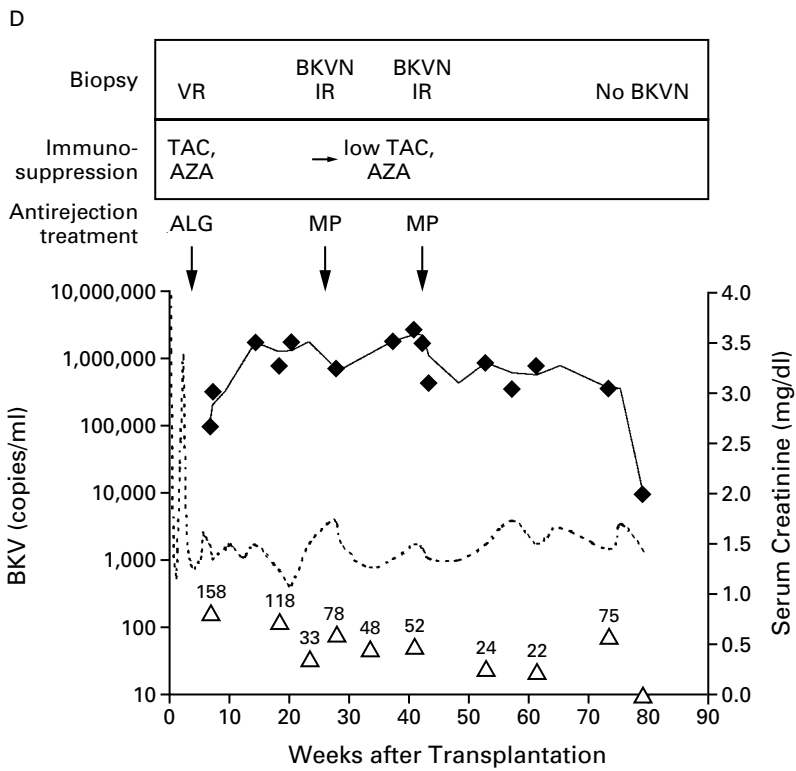
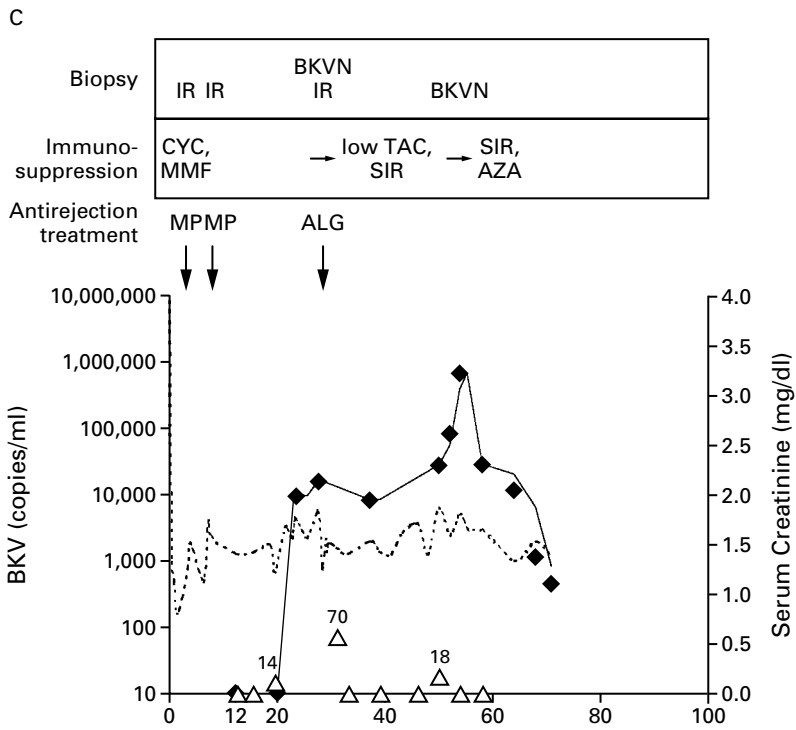
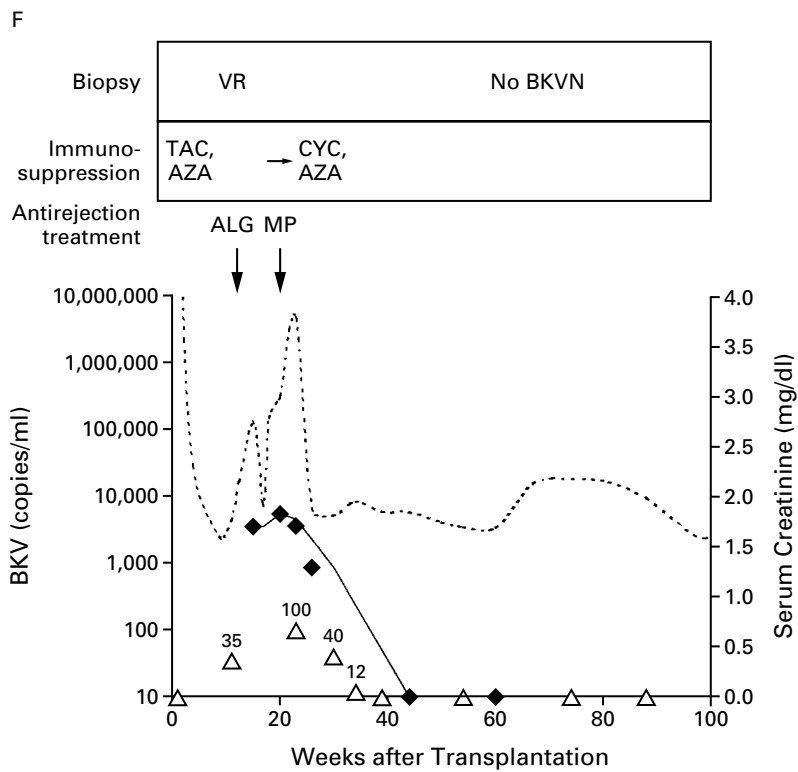
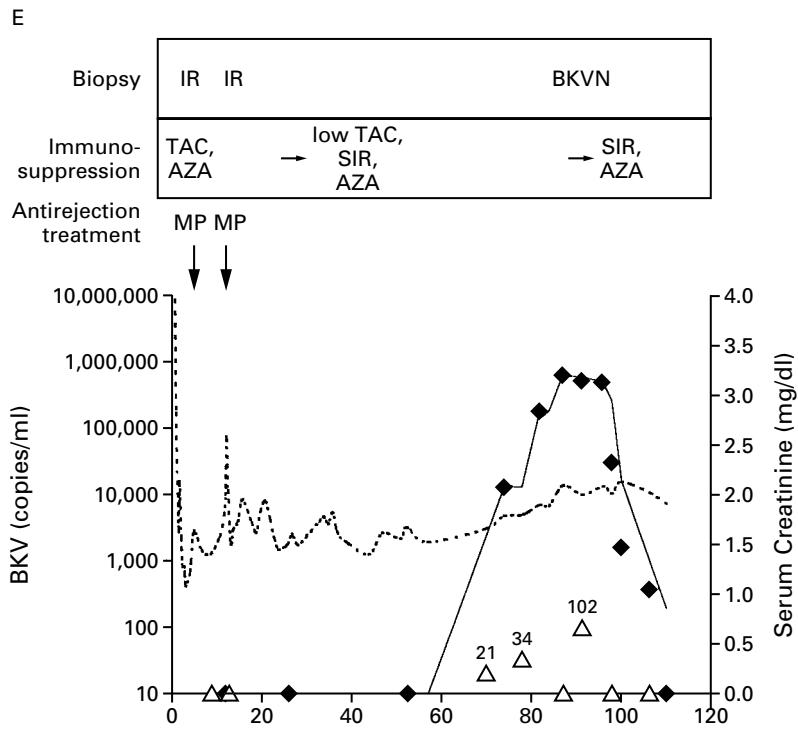


Figure 3. BK Virus (BKV) Levels in Five Patients with BKV Nephropathy (Panels A through E) and One with Viremia in the Absence of Nephropathy (Panel F).

Diamonds indicate BKV levels in plasma, triangles decoy cells in urine (the numbers denote the numbers of cells per 10 high-power fields), circles cytomegalovirus levels, and broken lines serum creatinine levels. Horizontal arrows indicate changes in the immunosuppressive regimen. VR denotes vascular rejection, BKVN BKV nephropathy, IR interstitial rejection, CYC cyclosporine, MMF mycophenolate mofetil, AZA azathioprine, TAC tacrolimus, low TAC low-dose tacrolimus (trough blood level, ≤ 4 ng per milliliter), SIR sirolimus, ALG antilymphocyte globulin, and MP intravenous methylprednisolone. To convert values for creatinine to micromoles per liter, multiply by 88.4.



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cell shedding), viremia, and nephropathy was 30 percent, 13 percent, and 8 percent, respectively. In line with previous data on the seroprevalence of BKV in adults, serum samples obtained before transplantation were positive for BKV in 77 percent of the transplant recipients.¹⁴ Four of five patients in whom BKV nephropathy developed were seropositive before transplantation, as were seven of eight patients in our previous retrospective study (unpublished data). Thus, BKV nephropathy constituted a secondary, rather than a primary, infection in most of our patients. These findings argue against the hypothesis that transplantation of kidneys from BKV-seropositive donors into BKV-seronegative recipients is a major cause of BKV nephropathy. However, the possibility that donor-derived viral strains have a role cannot be ruled out.

Routine screening for the presence of decoy cells in urine, a sign of enhanced polyomavirus replication in the renourinary tract, proved to be simple and was 100 percent sensitive, but only 71 percent specific, for the diagnosis of BKV nephropathy. Because of the low positive predictive value of decoy-cell shedding (29 percent), BKV viremia was evaluated as a diagnostic marker. Quantification of BKV viremia revealed that the viral load in plasma increased to more than 7700 copies per milliliter in all patients who had histologic evidence of BKV nephropathy. The viral load remained substantially lower in patients who had viremia without detectable BKV nephropathy in samples from allograft biopsies. Although BKV replication in the allograft cannot be ruled out in these patients, if present, it must have been limited or restricted to foci²² in order to be missed on renal biopsy and was therefore presumably less likely to cause allograft dysfunction. The correlation between the viral load and allograft involvement further suggests that BKV viremia in plasma is due in large part, if not entirely, to replication in the transplanted organ. This notion is supported by the rapid drop in the viral load in patients who underwent nephrectomy, despite the continued administration of maintenance immunosuppressive therapy.^{15,19}

A limitation of our study is the surprisingly small number of patients in whom BKV replication and nephropathy developed despite treatment with regimens containing tacrolimus or mycophenolate mofetil — drugs that presumably confer a susceptibility to BKV nephropathy. Nevertheless, our findings point to a pivotal role of rejection and its treatment in the pathogenesis of BKV nephropathy in patients receiving either tacrolimus or mycophenolate mofetil. The presence of decoy cells in urine and viremia, as measured by plasma PCR assays, may serve as noninvasive markers of BKV replication. These markers may be useful in identifying patients at risk for nephropathy and tailoring immunosuppressive therapy for such patients.

Supported by a Swiss National Fund Grant (3200-62-02.00).

Presented in part at the 11th European Conference of Clinical Microbiology and Infectious Diseases, Istanbul, Turkey, April 1–4, 2001.

We are indebted to Ms. V. Brombacher for excellent technical assistance and to Drs. V. Nickenleit, G. Thiel, M. Battagay, J. Schifferli, and N. Gyr for helpful discussions and generous support.

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