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Evaluation of a Preemptive Strategy for BK Polyomavirus-Associated Nephropathy Based on Prospective Monitoring of BK Viremia: A Kidney Transplantation Center Experience

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ABSTRACT

Introduction. BK polyomavirus-associated nephropathy (BKPVAN) is a major cause of renal failure early after kidney transplantation. The present study reports the preliminary results of prospective monitoring including a preemptive strategy for BKPVAN during the first year after kidney transplantation.

Methods. We monitored BK virus DNA in blood at months 1, 2, 3, 6, 9, and 12 among 92 subjects who received induction therapy (basiliximab or antithymocyte globulin), and maintenance immunosuppression with prednisone, mycophenolate mofetil, and tacrolimus. Patients with two or more consecutive measurements of viral load $>10^4$ copies/mL were treated with a stepwise approach including dose reduction or discontinuation of mycophenolate mofetil eventually followed by reduction of tacrolimus and introduction of leflunomide.

Results. Within 1 year, seven (7%) patients displayed sustained BK viremia at a median of 92 days after transplantation. Among 68 patients who underwent a renal allograft biopsy, seven were diagnosed as BKPVAN at a median of 15 weeks after transplantation. The diagnosis was achieved by a surveillance biopsy in four patients with stable renal function. BKPVAN was preceded by asymptomatic viremia except for two cases in whom BK viremia occurred at 6 or 11 months, after the histological diagnosis. At 12 months, six patients had cleared their viremia. Serum creatinine levels had stabilized in six recipients with BKPVAN estimated renal function was 43.7 ± 16.3 mL/min in patients with viremia and/or BKPVAN versus 61.3 ± 20.1 mL/min among patients who never became viremic ($P = .03$). None of the patients with viremia and/or BKPVAN lost the allograft.

Conclusion. BKPVAN may occur early after kidney transplantation, at a low or undetectable viremia or at some weeks after the first positive viremia. Intensive monitoring during the first 4 months after transplantation together with early protocol biopsies or interventions prompted by BK viremia may optimize BKPVAN diagnosis at a subclinical stage, thus avoiding renal dysfunction.

BK POLYOMAVIRUS-ASSOCIATED NEPHROPATHY (BKPVAN) affects approximately 1% to 10% of renal transplant recipients leading to as much as 60% of irreversible graft losses within 5 years.[1] The current recommendation includes screening for BK virus (BKV) reactivation with subsequent preventive reduction of immunosuppression with or without antiviral therapy.[1,2] Among available diagnostic methods for BKV infection, a BKV DNA load of $>10^4$ copies/mL plasma has been shown to have the highest predictive value for BKPVAN.[1]

We performed a 1-year prospective study to assess the incidence and kinetics of BKV replication in adult kidney

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transplant recipients. We evaluated the impact of preemptive reduction of immunosuppression combined with the antiviral agent leflunomide on viral load kinetics and the course of BKPVAN.

PATIENTS AND METHODS

Patient Population

This prospective study was conducted among 94 consecutive adult patients who underwent kidney (with or without pancreas) transplantation between February 2008 and May 2009. One patient was excluded due to a nonfunctioning allograft and one patient, due to poor protocol compliance, leaving 92 patients available for the analysis. The subjects had been prospectively followed over the first year after transplantation.

The evaluated data included donor and recipient demographic features and clinical characteristics of age, race, sex, blood group, cytomegalovirus (CMV) serological status, human leukocyte antigen (HLA) phenotype and match, renal failure cause, as well as transplant type. At each visit, we recorded the serum creatinine level and current immunosuppressive regimen. During the study period, we documented all episodes of acute rejection, CMV infection, or urinary tract infection as well as the onset of posttransplant diabetes.

Immunosuppressive Therapy

All the patients received induction immunosuppressive therapy with basiliximab or antithymocyte globulin (ATG), and maintenance therapy with prednisone (100%), tacrolimus, and mycophenolate mofetil (MMF; 97%). A switch to a sirolimus-MMF-based regimen was performed in 11 patients with stable renal function, during the second semester. Presumed or confirmed acute rejection episodes were treated with methylprednisolone (250 mg for 4 days, followed by gradual reduction in steroids) or with ATG, if necessary.

CMV Prophylaxis

CMV D^+/R^- received valganciclovir (900 mg orally once daily; doses adjusted on the basis of renal function) for 3 months after transplantation. In addition, valganciclovir was given for 1 to 3 months to CMV R^+ recipients who received ATG and to all recipients (except for CMV R^-/D^-) who were treated for acute rejection. The detection of CMV in blood or other body fluid specimens was defined as CMV infection.

BKV Monitoring

Blood samples for quantification of BKV load were collected at median times of 1, 2, 3, 6, 9, and 12 months after transplantation. BKV DNA copies were measured by real-time quantitative polymerase chain reaction (PCR). BK viremia was defined as positive detection of BKV DNA in plasma. Sustained viremia was defined as two or more consecutive positive plasma samples (over ≥3 weeks) in contrast with "transient" viremia.

Allograft Biopsy and Diagnosis of BKPVAN

During the first year, recipients underwent an allograft biopsy as part of a surveillance protocol or if clinically indicated due to an increased creatinine and/or elevated sustained BKV load. The clinical diagnosis of BKPVAN was confirmed by histological findings in renal biopsies: intratubular viral inclusions with nuclear staining positively for simian virus 40.

Management Protocol

BKV preemptive therapy. Patients with stable renal function and two or more consecutive positive viral load tests (>1000 copies/mL), were treated with a stepwise decrease in immunosuppression starting with MMF to 50%. In absence of a decrease in viral load over the next 2 weeks, MMF was discontinued and leflunomide introduced, as the second step. If BK viremia persisted, the calcineurin inhibitor was reduced by 15% to 20%, according to concentrations, as a third step. The daily dose of prednisone was 10 mg or less.

BKPVAN treatment. Patients with BKPVAN were treated with MMF discontinuation, reduction of the anticalcineurin agent by 20%, and institution of leflunomide. Patients with BKPVAN and concomitant acute rejection episodes received pulse steroids for the rejection and subsequently BKPVAN treatment, as described above.

Leflunomide treatment. Leflunomide was administered at an initial dose of 100 mg per day for 5 days, followed by maintenance doses between 20 and 60 mg/d. Blood levels of the active metabolite A77 1726 were measured at days 10, 20, and 30 as well as every 2 months after treatment initiation. The target blood level was 50 to 100 μg/mL. If tacrolimus continued to be prescribed the target tacrolimus blood level was decreased to 4 to 6 ng/mL.

Statistical Analyses

Data were expressed as mean values ± standard deviations or as medians and ranges, as appropriate. Differences between the groups were assessed by univariate analyses using Fisher exact test for categorical variables and Student t test for continuous data. Statistical significance was set at $P \leq .05$.

RESULTS

Patient Characteristics

Table 1 shows the demographic and clinicopathologic features of the 92 patients.

Incidence and Timing of BK Virus

Among 552 total possible samples from 92 recipients, 495 (89%) were collected during the first year after transplantation. BK viremia, detected in 21 (22%) individuals during the first year, was sustained in seven patients (7%). Median onset of viremia was 6 months: 3 months for sustained viremia (range = 1–12) and 6 months for transient viremia (range = 2–12). BK viral levels varied over a wide range among recipients with transient viremia. The median level was 2.32×10^5 copies/mL (range = 1.2×10^2–1.42×10^6). The median peak level was 5.33×10^4 copies/mL (range = 1.14×10^2–2.68×10^6) among recipients with sustained viremia.

BKV Nephropathy

During the first year after transplantation, 68 recipients underwent an allograft biopsy either within a protocol of surveillance (60%) or as clinically indicated. Seven of the 68 patients were diagnosed with BKPVAN at 4 months (range = 79–243 days). We diagnosed four cases of BKPVAN upon the protocol biopsy in the absence of graft dysfunction. BKPVAN was preceded by a period (60 ± 31 days; range = 8–180) of asymptomatic viremia

Table 1. Characteristics of the Study Population and Univariate Analysis of Patients With BKPVAN or BKV Infection and Patients Without BKV Infection

Characteristics	All Patients (n = 92)	Patients with BKPVAN or BKV Infection (n = 8)	Patients without BKV Infection (n = 84)	P Value
Recipient age, y (mean ± SD)	47.21 ± 12.2	50.88 ± 12.22	48.36 ± 12.28	.3621
Male sex (%)	61	37	64	.26
Causes of end-stage renal disease (n)				
Glomerulonephritis	43	3	40	
Polycystic kidney disease, dysplasia	25	2	23	
Vascular disease	5	0	5	
Tubulointerstitial disease	3	2	1	
Miscellaneous/others	15	1	14	
Dialysis (n)				
No dialysis	10	0	9	.72
Dialysis	82	8	75	
Hemodialysis	70	6	65	.87
Peritoneal dialysis	13	2	10	.61
Duration, mo (mean ± SD)	40.11 ± 29.86			
HLA mismatch <3 (n)	37	3	34	.86
Transplant organ (n)				
Kidney	85	8	77	.87
Kidney and pancreas	7	0	7	
Graft number (n)				
First/second/fourth	83/8/1	6/2/0	77/6/1	.17
Donor type (deceased/living)	77/15	6/2	71/13	.61
Donor age, y (mean ± SD)	44.55 ± 14.53	46.63 ± 9.53	44.36 ± 14.95	.14
Donor male sex (%)	51	50	52	.89
Cold ischemia time, min (mean ± SD)	627.34 ± 323.5	640 ± 425.9	626 ± 315.2	.9
CMV serostatus (n)				
D^-/R^-	31	4	27	.43
D^-/R^+	13	1	12	.88
D^+/R^-	21	3	18	.37
D^+/R^+	27	0	27	.09
CMV prophylaxis (n)	34			
Immunosuppressive treatment (n)	61/31	6/2	55/29	.71
Induction (basiliximab/ATG)	90	8		
Tacrolimus + MMF + prednisone		8		
DGF (n)	15	1	14	.76
Acute rejection				
≥one episode	21	3	18	.37
>one rejection	9	2	7	.17
Diabetes after transplantation (n)	20	3	17	.36
CMV infection (n)	17	1	16	
Urinary tract infection (n)	30	3	27	.97
eGFR, mL/min (mean ± SD)				
At 1 mo (MDRD)	52.66 ± 18.38	49.57 ± 2.01	52.94 ± 9	.64
At 12 mo (MDRD)	59.93 ± 20.33	43.71 ± 16.29	61.28 ± 20.12	.02
MDRD at 12 mo/MDRD at 1 mo < 80% (n)	8	1	7	.9

BKPVAN, BK polyomavirus-associated nephropathy; BKV, BK virus; SD, standard deviation; ATG, antithymocyte globulin; MMF, mycophenolate mofetil; CMV, cytomegalovirus; DGF, delayed graft function; eGFR, estimated glomerular filtration rate; MDRD, Modification of Diet in Renal Disease formula; HLA, human leukocyte antigen; D, donor; R, recipient.

(median viral load = 2×10^5 copies/mL; range 1.37×10^4–2.68×10^5 at the time of the biopsy) except for two cases of BK viremia at 6 and 11 months after the diagnosis of BKPVAN. JC viremia was not detected in these recipients. In one patient, a biopsy performed at 1 month after the first step to reduce immunosuppression and before introduction of leflunomide showed features of an acute rejection episode.

Clinical Correlations

There was no significant association with detection of BKV in plasma among demographic variables (recipient age and sex, HLA mismatch, donor age and sex donor or recipient, CMV serological status cold ischemia time, cadaveric versus living donor transplant) or posttransplantation events (acute rejection episodes, CMV infections, urinary tract

infections, or occurrence of posttransplant diabetes). There was also no significant association between the immunosuppressive regimen and the BKV load (Table 1).

Clinical Follow-up of Patients With BKV Viremia and/or BKPVAN

All patients with BKPVAN underwent reduction of immunosuppression and treatment with leflunomide. Thereafter, BK viremia declined to undetectable levels in the six patients at a median of 4 months (range = 2–9). One patient with low-level BK viremia (maximal level = 2.68×10^5 copies/mL) did not have any change in immunosuppressive treatment and did not receive leflunomide. The viremia resolved in 4 months.

At 12 months, the estimated renal function displayed by affected patients was 43.7 ± 16.3 mL/min versus 61.3 ± 20.1 mL/min among patients who never experienced viremia ($P = .03$). One patient developed renal failure after the diagnosis of BKPVAN. He never achieved leflunomide levels in excess of 40 μg/mL because of side effects of this drug. Leflunomide was stopped during the second year, namely, 8 months after its introduction, and tacrolimus was switched to cyclosporine. Viremia then decreased to $\leq 10^4$ copies/mL and the serum creatinine stabilized. In the other six patients with BKPVAN, reduction of immunosuppression associated with leflunomide therapy stabilized allograft function: namely, 55.5 ± 0.9 mL/min at the time of biopsy to 59.5 ± 4.5 mL/min at the end of the first year among recipients diagnosed with BKPVAN by surveillance biopsy, and from 33.04 ± 3.22 mL/min to 34.25 ± 1.25 mL/min among these diagnosed due to an increased serum creatinine.

No patient experienced an acute rejection episode after introduction of leflunomide. None of the patients with viremia and/or BKPVAN lost their renal allograft during the first year after transplantation.

DISCUSSION

Prospective surveillance in our center using plasma and biopsy detection demonstrated the presence of detectable viremia that was managed with leflunomide and reduction of maintenance immunosuppressive treatment. Viremia was sustained in 7% of recipients, generally occurring in the first 4 months after transplantation. This incidence is similar to those reported by centers using intensive monitoring during the first trimester.[3,4] As illustrated in our study and in other reports,[3,4] BKV replication occurs early after transplantation.

BKPVAN was diagnosed in seven patients (7.6%) at 4 months after transplantation. In four cases, nephropathy was detected at a subclinical stage owing to protocol biopsies. As previously shown,[5,6] surveillance allograft biopsies may help to identify early disease with minimal histological changes and to improve short- and long-term graft survivals. Except for the two patients with BKPVAN, the histological diagnosis was associated with viremia exceeding 10^4 copies/mL, an observation that is consistent with the recommendation to perform an allograft biopsy when the plasma BKV load is above 10^4 copies/mL.[1,3,7] BK viruria appears to precede the development of BK viremia by a median of 4 weeks and histologically proven BKPVAN, by a median of 12 weeks. The high negative predictive value of 100% of assays for viruria and viremia allow the clinician to rule out BKPVAN, given a negative test result. Persistent BKV DNA viral loads of more than 10^4 copies/mL of plasma for ≥4 weeks had been shown to be associated with an increased probability of histologically proven BKPVAN; sensitivity 93% and specificity 93%[1] However, these results must be interpreted with caution for most of them were established among cases of BKPVAN with graft dysfunction and because there is intra- and interlaboratory variability of polyomavirus PCR assays due to incompletely standardized differences in primers, probes, and viral control reagents.

These data may partly explain the rare cases of BKPVAN that show no plasma BKV load, as reported in the literature[8,9] and noted in two of our study patients. Another explanation may be the involvement of another type of polyomavirus such as JC virus (JCV), which can cause nephropathy. JC nephritis is known to share the same cytological, histological, and nuclear SV 40 staining features with BKV nephritis. JCV and BKV nephropathies are distinguished by molecular tests.[10] In our study, JCV was negative when BKV was detected in blood.

Regarding the efficacy of our BKV screening protocol, most patients with sustained viremia and/or BKVNA were treated with leflunomide and reduction of immunosuppressive treatments: namely, MMF discontinuation and tacrolimus reduction. Leflunomide has been chosen for its immunosuppressive and antiviral properties.[11–13] Maintenance of global immunosuppression may be important in the first semester following transplantation, a high-risk period for rejection. This therapeutic approach was followed by a slow reduction of viral replication and stabilization of renal function in most treated subjects. Of note, the only patient who developed renal failure after the diagnosis of BKPVAN failed to achieve plasma concentrations of leflunomide greater than 40 μg/mL during 7 months. The "therapeutic" leflunomide (or more precisely A77, 1726) level is still debated, because of the risk of toxicity.[14] Due to the wide pharmacokinetic variations of leflunomide used at high doses for BKV infection treatment, monitoring of the active metabolite is strongly recommended.[15] Some authors[16,17] have reported better outcomes among patients who display A77, 1726 concentrations >40 μg/mL. The 40 μg/mL target level was derived by extrapolating in vitro effective concentrations.[16] Hemolysis markers have been observed for leflunomide levels at 81.4 ± 14 μg/mL.[14] According to Williams et al,[15] hematologic and hepatic side effects are noted in 25% to 35% of patients whose serum levels are above 100 μg/mL and no rejection episodes occurred when the serum levels of A77, 1726 were above 50 μg/mL. In our study, no patient developed an acute rejection episode after the therapeutic intervention. Encourag-

ing results have been described in retrospective[14,18,19] and prospective[16,17,19] small series of BKPVAN treatment with leflunomide. However, in a systematic review of these reported cases, Johnston et al[20] could not demonstrate a graft survival benefit deriving from the addition of leflunomide. Whether reduction of immunosuppression alone would stabilize allograft function in these patients is unknown, due to the lack of a randomized study. Moreover, the long-term effects of leflunomide and the ideal duration of this therapy for BKV infection are unknown.

The preliminary results of this prospective study indicated that BKV replication and occurrence of BKPVAN were early events after renal transplantation in our center. Prompt diagnosis and intervention combining leflunomide and reduced immunosuppressive treatment are crucial to stabilize renal function. Given our initial results, we believe that a greater number of early protocol biopsies might allow us to better define thresholds of our PCR assays with regard to the diagnosis and the intervention. Furthermore, larger patient populations and longer follow-up are necessary to document the risk factors for BKV infection among our transplant population and to assess the benefits as well as long-term effects of leflunomide therapy for renal transplant patients with BKPVAN.

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