CORRESPONDENCE

Prospective Monitoring of BK Virus Load after Discontinuing Sirolimus Treatment in a Renal Transplant Patient with BK Virus Nephropathy

To the Editor—The polyomavirus BK may cause progressive renal allograft failure in 1%–5% of kidney transplant recipients [1, 2]. The risk factors for BK virus nephropathy (BKVN) are not well understood but may involve rejection episodes and treatment with antilymphocyte preparations and potent new immunosuppressive drugs such as tacrolimus and mycophenolate mofetil [3]. Diagnosis of BKVN requires the immunohistochemical demonstration of BK virus–infected tubular epithelial cells in the allograft biopsy specimen. We note that detection of BK virus DNA in plasma closely follows the course of BKVN and may serve as a noninvasive tool for diagnosis and monitoring [4]. Recently, Limaye et al. [5] confirmed our findings in a retrospective study of 4 patients with BKVN and showed that the BK virus load decreased when the immunosuppressive treatment was reduced or when the renal allograft was removed.

We have independently established a quantitative assay of a similar format (real-time fluorescent probe–based assay [TaqMan, Applied Biosystems]: primer 1, AGCAGGCAAGGGTTCTATTACTAAAT; primer 2, GAAGCAACAGCAGATTCTCAACA; probe, AAGACCCTAAAGACTTTCCCTCTGATCTACACCAGTTT labeled with 6-carboxyfluorescein at the 5′ end and 6-carboxytetramethylrhodamine at the 3′ end), which allows the detection of BK virus over a linear range of 10–10^7 copies per reaction. We prospectively followed a 65-year-old renal transplant recipient who developed BKVN in January 2001 while on an immunosuppressive treatment with cyclosporine, prednisone, and sirolimus. The underlying diagnosis of mesangioproliferative glomerulonephritis was made in 1987. He began hemodialysis in 1999 and received a renal transplant from a cadaver in October 2000. As shown in figure 1, initial allograft function was good (creatinine clearance, 55 mL/min). Two months after transplantation, a rise in serum creatinine from 1.52 mg/dL (135 μmol/L) to 2.47 mg/dL (218 μmol/L) was noted. Sirolimus concentrations (mean ± SD) were 5.8 ± 2.4 ng/mL (median, 6.4 ng/mL; range, 3.0–6.6 ng/mL), and blood levels of cyclosporine (mean ± SD) were 242 ± 108 ng/mL (median, 225 ng/mL; range, 122–324 ng/mL).

In the first allograft biopsy, a mononuclear and plasmacytoid interstitial infiltration was seen together with an altered tubular epithelium, and acute rejection was diagnosed. Intravenous methylprednisolone (1000 mg for 3 days) was given, but renal function did not improve. A second transplant biopsy 2 weeks later revealed more-pronounced tubular alterations. BKVN was diagnosed by immunohistochemistry. BK virus DNA was detected in plasma, and the BK virus load was 3×10^6 copies/mL. Sirolimus therapy was stopped; however, prednisone (15 mg/day) and cyclosporine therapy was continued (blood levels [mean ± SD], 156 ± 26 ng/mL; median, 153 ng/mL; range, 76–121 ng/mL). Over the next 12 weeks, the BK virus load decreased until it was no longer detectable (figure 1). In parallel, allograft function improved, with a serum creatinine concentration of 1.74 mg/dL (154 μmol/L).

We believe that this case is the first description of BKVN while the patient was undergoing immunosuppressive triple therapy with sirolimus. Of note, the sirolimus concentrations had not been high nor had there been prior treatment with antilymphocyte preparations. BKVN must be distinguished from interstitial rejection, which may coexist at times [6]. In the absence of rejection, treatment with steroids does not result in a functional or histologic improvement [1, 2]. Because specific antiviral treatment is not established, reduction of immunosuppression may be an option if carefully monitored [4]. In our patient, sirolimus therapy was stopped while cyclosporine and prednisone therapy was continued at slightly lower levels. Prospective monitoring of the BK virus load revealed a decrease by 5 orders of magnitude over 12 weeks. This, together with the improving allograft function, supported the critical decision to reduce the immunosuppressive treatment.

The patient's BK virus load was 3–4 orders of magnitude higher than the levels determined by Limaye et al. [5]. This difference in BK virus load cannot be attributed to sirolimus, because we found similarly high BK virus loads in 9 patients undergoing treatment with tacrolimus, a finding that we confirmed independently in 2 cases by limiting-dilution polymerase chain reaction (PCR) [4]. In 2 of 41 control patients without BKVN, we found BK virus loads of 511 and 748 copies/mL [4]. In fact, we have never observed histologically manifest BKVN at BK virus levels of <5000 copies/mL (authors' unpublished

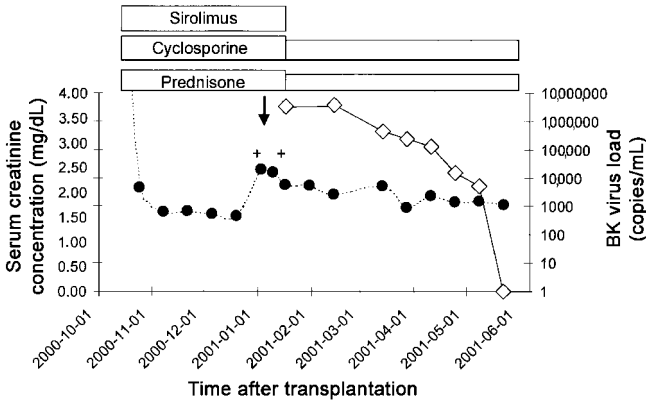

Figure 1. Time course (year-month-day) of serum creatinine concentration, immunosuppression, and BK virus load. *Circles,* serum creatinine concentration; *diamonds,* BK virus load; +, allograft biopsy; *arrow,* steroid pulse; *boxes,* duration of treatment with sirolimus, cyclosporine, and prednisone.

data). The reason for the difference in BK virus load levels is not clear to us. The target sequences used in the respective real-time PCR assays are not identical but overlap significantly in the gene coding for the BK virus large T antigen. In both assays, sample DNA is added to a master mix (5 μL of 25 μL in our assay vs. 10 μL of 50 μL in the assay used by Limaye et al. [5]). Both groups use silica spin columns (Qiagen) for DNA preparation. Elution of the DNA resulted in a 2-fold concentration in our procedure, compared with a 4-fold concentration in the assay used by Limaye et al. [5]. Thus, inhibition of the real-time PCR assay owing to a high DNA concentration does not seem likely. Because of the implications of the absolute BK virus load for diagnosis and for monitoring, the differences should be communicated and hopefully resolved.

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Reply

To the Editor—We appreciate the interest of Hirsch et al. [1] in our recent study of quantitation of BK virus DNA in the blood of patients with histologically documented BK virus nephropathy (BKVN). In our study, we retrospectively analyzed serum samples from 4 renal transplant patients with BKVN [2] and showed that BK virus DNA appeared in blood weeks to months before the histologic diagnosis of BKVN in renal biopsy specimens. BK virus DNA was not detected in the blood of matched control patients who did not develop histologic evidence of BKVN. Furthermore, the levels of BK virus DNA in blood decreased and/or became undetectable among patients who had improvement in renal function or who underwent transplant nephrectomy. We concluded that quantitation of BK virus DNA in blood was useful for both the diagnosis and monitoring of patients with BKVN.

Hirsch et al. [1] confirmed our findings by using a different assay with a similar format (real-time, quantitative polymerase chain reaction [PCR] with different target sequences) for a patient with histologically documented BKVN [1]. The BK virus DNA load in blood at the time of diagnosis of BKVN in the patient was 3×10^6 copies/mL plasma and eventually became undetectable over the next 12 weeks, as immunosuppression was reduced. Overall, the results from the two reports are similar and provide evidence that BK virus DNA levels in blood reflect the degree of renal involvement with BK virus. These studies also suggest that monitoring BK virus load in blood may be potentially useful both for identifying patients at risk for developing BKVN and for monitoring response to therapy (reduction in immunosuppression) among patients with BKVN.

The finding that the absolute BK virus load in blood was significantly different between the two assays is not surprising and highlights the difficulties in interpreting results of PCR assays between different laboratories, as documented for various other viral pathogens. In our study, the BK virus load in the blood of patients with BKVN ranged from 530 to 1.8×10^4 copies/mL, which was significantly lower than the virus load of the patient reported by Hirsch et al. [1]. In addition, Hirsch et al. report similarly high levels in other patients with BKVN, although they did not provide specific details. Although similar PCR methodologies (extraction and real-time quantitation) were used in the two studies, different target sequences were used. Other factors that could potentially explain the differences in the absolute BK virus load in the two studies include differences in immunosuppression (specific agents and target levels), patient population (mostly kidney/pancreas transplant recipients in our study vs. kidney transplant recipients in the study by Hirsch et al.), extent of BK virus nephropathy, and type of specimen used (serum vs. plasma). Perhaps the most important difference between the studies was the timing of blood samples relative to the diagnosis of BKVN. In our study, the median timing of the blood samples was 26.5 weeks (range, 5–69 weeks) before the histologic diagnosis of BKVN, whereas the majority of patients in the study by Hirsch et al. appear to have had blood samples analyzed within a few weeks of the histologic diagnosis of BKVN. It is possible that the BK virus load increased several log-fold in the relatively long duration between the timing of the blood samples and the diagnosis of BKVN in our study. Given the retrospective nature of our study, we are unable to test this hypothesis by analyzing additional blood samples obtained closer to the histologic diagnosis of BKVN.