

Detection and Viral Load Monitoring of BK Virus in Hemorrhagic Cystitis Complicating Bone Marrow Transplant Patients

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Hemorrhagic cystitis (HC) is an important cause of morbidity and mortality in patients undergoing bone marrow transplantation (BMT). The manifestations vary from microscopic to gross hematuria and may be associated with obstructive uropathy and renal failure secondary to clotting in the urinary tract. Its incidence has varied from 7% to 68% of BMT cases. HC can also be due to the toxic effects of drugs used in the BMT conditioning regimen. Such as cyclophosphamide (Cy). Other risk factors for HC include the use of busulfan and pelvic irradiation, older age at transplantation, allogeneic BMT, and graft-versus-host disease (GVHD).

The polyoma virus BK (BKV) was observed in early studies to be associated with the development of HC in BMT patients. Recent studies with highly sensitive polymerase chain reaction (PCR) showed that BKV could be detected in the urine of BMT patients with or without HC. This is probably owing to the fact that, after primary infection, BKV remains dormant in the uroepithelium and the immunosuppression associated with BMT leads to reactivation of viral replication that results in viruria and at times viremia (Figure 1). Thus it is likely that HC is more likely to be correlated with degree of viruria rather than just the presence or absence of viruria.

Recently, quantification of viral DNA load has been reported to be a valuable tool to monitor various infections. Use of real-time, quantitative, Polymerase Chain Reaction (RQ-PCR) technology allows accurate early detection as well as quantitation of viral loads. In this study, we tested the hypothesis that although BKV could be detected non-quantitatively by PCR in most BMT patients, a quantitative PCR method might allow identification of viral load that is better correlated with HC and thus aid in the diagnosis and management of serious BK virus infections in BMT.

Methods: We utilized the RQ-PCR technique (TaqMan) to quantify BKV DNA in clinical specimens using primers designed for a region of the VP-1 and Agno genes that are conserved. Additionally, to monitor the PCR product during amplification, we designed TaqMan probes that are complementary to the internal regions of the amplified products (Figure 2). We screened 62 BMT patients with HC. A total of 253 clinical specimens (129 blood, 124 urine) were screened. In addition 1 kidney tissue samples, 3 fluid what fluid samples were screened. Details of one patient who was serially followed.

Results: BKV was detected in 41 patients out of the total 62 patients tested. Of the 129 blood samples, 68 tested positive for BKV. The blood viral load range was 400-1.9x10⁷copies/ml. Similarly, of the 124 urine samples, 100 tested positive. The urinary viral load range was 100-10⁹copies/ml (Figure 3).

One 15-year old male patient with unrelated donor marrow transplant for paroxysmal nocturnal hemoglobinuria developed severe GVHD, and aspergillus and pseudomonas sinusitis. At 140 days post

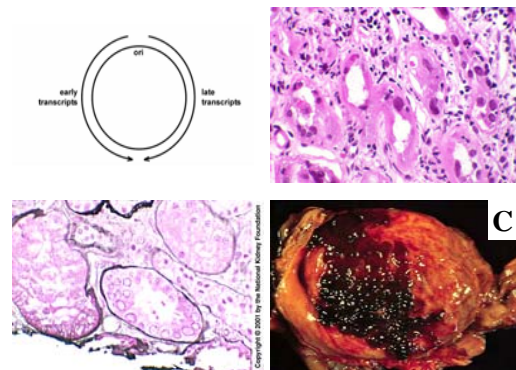


Figure 1: BK virus is a double stranded DNA virus with ~5.2kb long (A), normally latent in kidney. Infection occurs in transplant or other settings of immunosuppression. There is a pleomorphic interstitial infiltrate composed of lymphocytes and plasma cells with occasional admixed PMNs and characteristic viral inclusions in tubular epithelial cells, as shown here (B) and BK polyoma virus nephropathy may also show cleared viral inclusions in tubular cells, rather than the enlarged, basophilic viral changes (C) in kidney transplant patients. It also causes number of clinical symptoms, of which hemorrhagic cystitis (D) is commonly seen in BMT patients.

transplant, he developed pancytopenia and sinopulmonary disease along with hemorrhagic cystitis. He tested BKV-positive in blood and urine and was followed serially. No CMV, EBV or adenovirus could be detected in this patient. He had persistently high levels of BK virus in the urine (1.2-1.3x10⁹copies/ml) despite clearing BKV from the blood (with an initial viral load of 1.4x10⁶copies/ml). However, on further testing, BKV was found in bone marrow (6.5x10⁴ copies/ml), tracheal aspirate (6.1x10⁴ copies/ml), and lung tissue (16 copies/cell) supporting progressive dissemination of the BK virus.

Conclusions: A large proportion (41 / 62 or 67%) of BMT patients with HC were found to have detectable but very widely variable BKV load in urine and / or blood as detected by the RQ-PCR assays. In addition to renal / urinary tract disease BKV can also cause disseminated disease that could also be diagnosed by this assay.

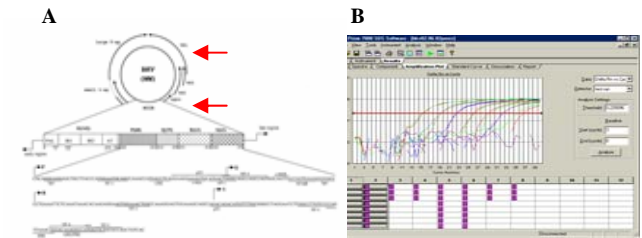


Figure 2: BK virus is double-stranded DNA virus with 5.2kb long (A). The genomic structure shows the direction of gene expression. To detect BKV, we designed primers and probe based on the conserved VP1 and Agno genes. The red arrows indicate the location of these genes, our detection targets for real time quantitative PCR. Above is a screen snap shot of real time fluorescent curves on an ABI SDS 7000 machine for BKV (B). The curves on the left represent amplification of higher copy numbers whereas curves on the right represent lower copy numbers.

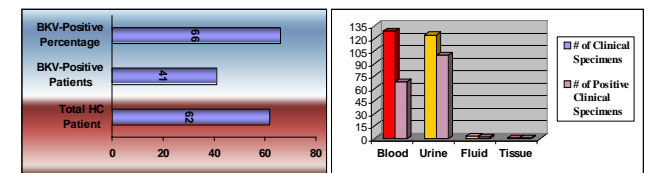


Figure 3: Clinical utility of real time quantitative PCR for detecting and monitoring BKV infection in bone marrow transplant. (Left) BKV was detected on 41 of 62 patients suffering from hemorrhagic cystitis. Additionally, BKV was detected in different types of clinical specimens, including blood, urine, other body fluids and tissue samples.

This assay, thus, can provide a sensitive, specific, reliable and rapid method for diagnosis of BKV infection in BMT patients. The quantitative viral load measures would be useful in guiding as well as monitoring the response to specific therapy.

