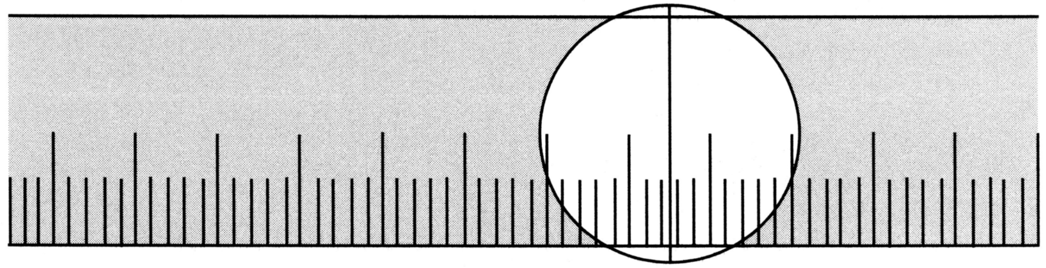
LAB NEWS



From the Department of Laboratory Medicine - Yale-New Haven Hospital Medical Center

Clinical Virology Laboratory Newsletter

Vol. 20 (1) **Jan. 2011**

BK Virus Nephropathy, Genome Variability and the Pitfalls of PCR Surveillance

BK virus nephropathy (BKVN). Polyomavirus BK was first isolated in 1971 from the urine of a kidney transplant patient with the initials B.K. (1). It is now known that primary infection occurs in childhood and is usually asymptomatic. The virus persists in kidneys and ureters and subclinical reactivation occurs with pregnancy, age and immune compromise. The main clinical complication of BKV is interstitial nephritis in renal transplant recipients (2). Possible risk factors for developing BK nephropathy after renal transplant are given in the Figure on page 2.

After kidney transplant, BK virus is detected in a stepwise fashion: first in urine in 30-60% of patients post-transplant, then in the blood in 10-15%, progressing to BKVN in 1-10%. BKVN damage progresses from limited focal disease to irreversible fibrosis and loss of the kidney in up to 60-70% of those affected.

Although definitive diagnosis of BV virus nephropathy is made by kidney biopsy and pathologic examination of tissue, BK viremia can be an early marker of involvement of the kidney (3). Since it is critical to detect BK virus nephropathy early and intervene before damage is irreversible, in 2005 an international panel of experts recommended surveillance by periodic quantitative BKV PCR in the first year after transplant (4).

Recommendations for early detection by PCR. Several algorithms exist for BKV monitoring. Some centers use an initial urine screen for decoy cells or BKV DNA, and if urine is positive, perform BKV PCR on plasma. At YNHH, surveillance for BKVN is by quantitative BKV PCR of plasma only. A plasma BK viral load of ≥10,000 copies/ml of plasma has the strongest positive predictive value for BKVN (table 1), and is an indication for kidney biopsy for definitive diagnosis.

Table 1: Diagnostic tests for BKVN

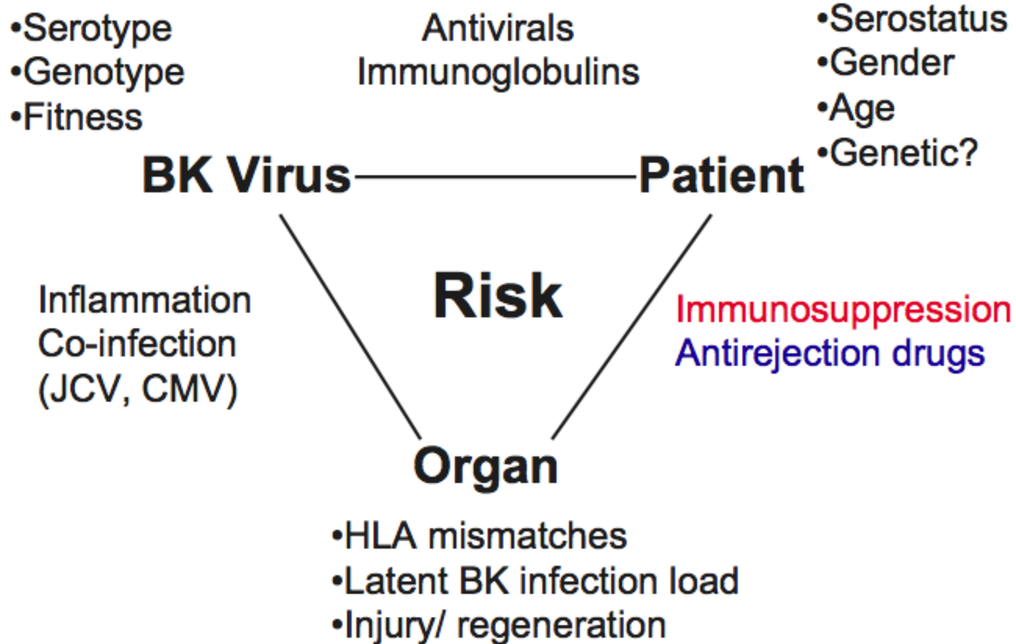
Test	Threshold	Correlation with BKVN+ Biopsy
Urine decoy cells	>10 cells per cytospin	+
Urine BK PCR	> 10^7 copies/ ml	++
Plasma BK PCR	> 10^4 copies/ ml	+++

A monoplex BKV PCR assay, developed at the NIH (5), was implemented by the Virology Laboratory in 2005. At defined time points post-transplant, plasma samples from renal transplant patients have been monitored using this BK PCR. Once diagnosed, the main treatment of BKVN is to reduce immunosuppression and allow the host's own immune system to resume effective control of BK virus.

Genetic variability leads to falsely low or negative viral loads. Early detection of BKVN relies on an accurate BK viral load of 10,000 copies/ml. However, BKV PCR assays vary between institutions and there is no international BKV DNA standard to benchmark quantification. Investigators at the University of Washington in Seattle evaluated 7 BKV PCR protocols used at major transplant centers and found marked variability (>1 log10) in 20% of the positive viral load results. Multiple BKV subtypes (Ia, Ic, II, III, IV, V, VI) exist and most current assays miss or under-quantitate subtypes III and IV. To address this problem, they devised a multiplex BK PCR assay using two gene targets (VP1 and T), two primers sets and three probes (6).

Over the past year, since becoming aware of this problem, the Clinical Virology Laboratory has scrutinized the amplification curves of our patients looking for indications of primer-probe mismatches. We identified 4 patients who were under-quantitated by the NIH assay. These patients' samples were sent to Seattle for accurate quantification until our laboratory could devote the time needed to validate and implement the Seattle assay in-house.

Notification of transition to multiplex BKV PCR assay at YNHH. On December 20, 2010 the Clinical Virology Laboratory transitioned from the NIH monoplex PCR to the Seattle multiplex BKV PCR assay. Validation studies in our laboratory indicated that viral loads of patients currently monitored using the NIH assay may be up to 0.4 log 10 higher with the Seattle multiplex assay. A comment including date of transition and effect on viral load will appear in the patient reports.



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References

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