

BK Virus Detection by PCR in Renal Biopsy Specimens from Renal Transplant Patients

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Abstract

Background: BK virus (BKV) infection in renal transplant patients is a constant concern. As BK-induced nephritis may lead to graft failure, detection of the virus is of significant clinical importance. While the diagnosis of BKV infection can be made on H&E stained slides, immunohistochemistry allows for greater detection. The goal of this study was to compare the effectiveness of PCR to conventional (Hematoxylin and Eosin [H&E]) and immunohistochemistry for BKV.

Design: Sixty-five renal biopsies from renal transplant patients were selected; 26 had definitive histologic and/or immunohistochemistry evidence of BKV and the remaining 39 cases lacked sufficient evidence. DNA extraction was performed and PCR was conducted. In addition, analysis was performed on 49 renal biopsies taken from nontransplanted patients to establish specificity.

Results: In the 26 cases with BKV infection by H&E/immunohistochemistry, PCR detected BKV in 10 cases (62% sensitivity). In the control group, no evidence of BKV was shown by PCR (100% specificity). Of the 39 cases with suspicion of BKV but negative H&E/immunohistochemistry, PCR found 2 cases with BKV. No viral inclusions were seen on H&E, but immunohistochemistry had not been performed. Subsequent immunohistochemistry showed focal BK positivity and

confirmed PCR detection.

Conclusions: These findings indicate that PCR is a highly specific detection method of BKV, although the sensitivity is low when compared to traditional H&E and immunohistochemistry methods. In light of these findings, the use of PCR may be particularly useful in cases with equivocal findings on H&E or immunohistochemistry.

Keywords: BK virus; PCR; Renal transplant; Formalin-fixed paraffin-embedded; Diagnostics

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Introduction

BK virus (BKV) is a member of the human polyomavirus family (*Polyomaviridae*), which also includes JC virus. They both have a high seroprevalence (as high as 80%) in the adult population and rarely cause any symptomatology in immunocompetent patients [1, 2]. BKV was first isolated in 1971 in a kidney allograft. It has since been determined to be the primary etiologic agent in what is now referred to as BKV-associated nephropathy (BKVAN) that almost exclusively exists

in renal transplant patients [1, 3]. In addition to this role, BKV has also been shown to be the etiologic agent of hemorrhagic cystitis in bone marrow allograft patients [4-7].

BKV-associated nephropathy is reported to occur in 1–10% of renal transplant patients, usually within the first year after the procedure [8]. Once the virus has reached certain level, up to 50% of patients will experience allograft failure [9]. Because of these serious consequences to BKVAN and that no specific antiviral treatment exists for the BKV, efforts were accelerated to find methods to screen for BK viral levels prior to development of BKVAN. Once it was determined that BKV infection viruria precedes detectable viremia, which precedes clinically evident BKVAN, polymerase chain reaction (PCR) tests have been utilized to quantitate viral loads in urine and/or plasma to help modulate immunosuppressive therapies before significant damage had occurred in the allograft [10].

Despite the clinical effectiveness of urine and plasma screening methodologies, renal needle biopsy still has a role in the evaluation. It allows for staging of cellular damage and can offer the added benefit of ascertaining any additional pathology that may be causing renal dysfunction outside of BKV. Current diagnostic practice employs Hematoxylin and Eosin (H&E) light microscopic examination, usually in conjunction with Immunohistochemistry (IHC) for BKV. While PCR methods have been widely studied on urine and plasma samples [11, 12], limited work has been done to evaluate the utility and feasibility on tissue specimens. In this study, we present our findings on using PCR detection method in cases of histologically proven BKVAN and a separate cohort of cases where BKVAN was suspected clinically, but not detected via conventional histology, to determine the sensitivity and specificity of PCR in this setting.

Materials

A search of our institution's surgical pathology case files was undertaken after approval by our Institutional Review Board. A total of 65 cases were identified and formalin-fixed, paraffin-embedded (FFPE) tissue blocks were retrieved. Two separate

subsets of cases were identified and isolated: 1) cases where definitive evidence of BKVAN was found by H&E and/or IHC methods (26 cases); and 2) renal allograft biopsy specimens where definitive evidence of BKVAN was not found by H&E with or without IHC (39 cases). In addition, a “control” population of 49 native renal biopsies performed for reasons not pertaining to diagnosing BKVAN and where suspicion of this entity was negligible was obtained.

Methods

All cases, including the control set, were subjected to PCR for BKV. For each block, 5 10µM thick scrolls of tissue were cut and DNA extractions were performed as per manufacturer's (QIAamp DNA FFPE Tissue Kit, Qiagen, Valencia, and CA) specifications. Extracted DNA was then amplified via in-house protocols used for BKV DNA detection in human plasma and urine by amplifying a 274 base-pair fragment of the *BKV VP2* and *VP3* genes. The assay was intended for quantitation and reported in DNA copies/mL. A threshold of 500/mL was used for positivity. Analysis occurred on the Light Cycler 2.0 (Roche Diagnostics, Indianapolis, IN). Results were recorded as either “positive” or “negative” for BKV.

Immunohistochemical staining for BKV was performed on all cases, as per manufacturer's instructions, where staining had not been performed as a part of the initial biopsy workup (Anti-BKV, 1:50, Chemicon, Billerica, MA).

Results

Sensitivity and Specificity of PCR

Sensitivity and specificity were calculated by using traditional histology, i.e., typical viral inclusions on H&E and/or positive for typical staining by BKV IHC, as the “true positives” with “true negatives” lacking both.

1) Sensitivity

There were 26 cases examined where BK virus was diagnosed by light microscopic methods. Typical morphologic features of BKV and immunohistochemical evidence were seen in all 26

cases [Figures A and B]. When looking at the results of our PCR assay, only 16 of these cases were found to be positive for BKV. These data result in a sensitivity of 62%.

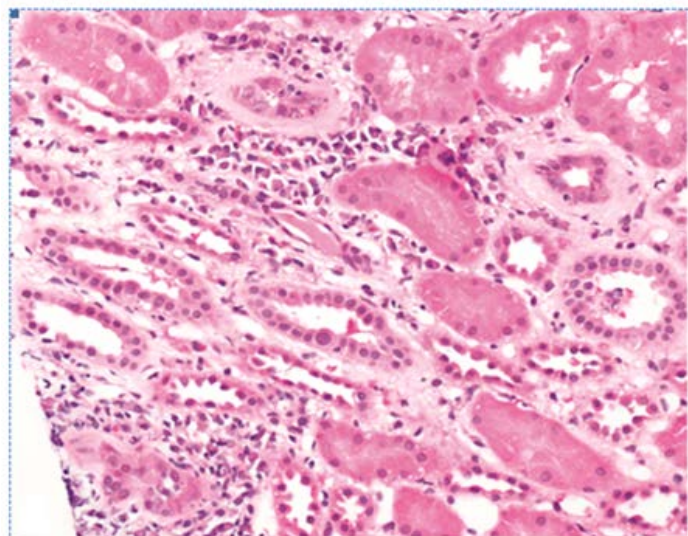


Figure (A): This renal biopsy micrograph (H&E, 400x) demonstrates tubular epithelial cells with nuclear changes consistent with BK viral infection. Although subtle, a significantly enlarged nucleus (*arrow*) towards the center of the field is found to have an intranuclear inclusion.

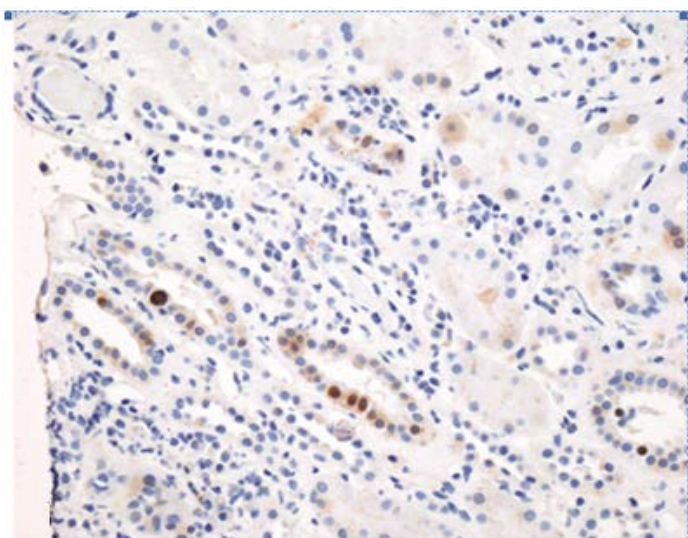


Figure (B): Immunohistochemistry for BK virus on the same case demonstrates strong nuclear reactivity in many of the tubular epithelial cells.

2) Specificity

Of the 49 cases designated in the control series coming from nontransplanted patients, no evidence of BKV was found via H&E, IHC, or PCR methods. These findings yielded 100% specificity for PCR when considering H&E/IHC as the gold standard in diagnosis.

PCR Result on Additional Histologically Negative Specimens

In these cases, definitive evidence of BKV was not found by either H&E or IHC methods in any cases and PCR was performed to determine if possible BKV was missed (false negatives). Of the 39 cases, 3 (8%) of cases were found to be positive for BKV by PCR. Subsequent review of these cases confirmed lack of evidence of BKVAN by H&E; however, it was noticed that weak, patchy reactivity to the BK IHC was present in all 3 of these cases [Figures C and D]. When classifying the PCR-positive cases as false negatives, this analysis yields a negative predictive value of 92% when considering H&E/IHC as the gold standard.

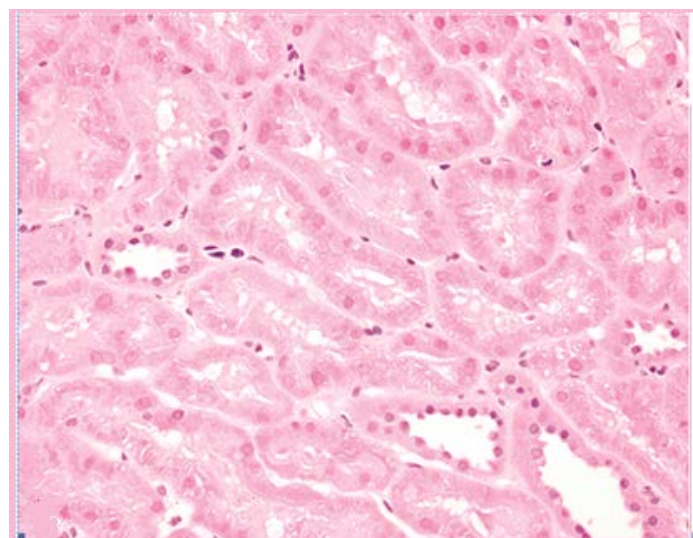


Figure (C): This renal biopsy (H&E, 400x) from one of our "equivocal" cases does not show any of the definitive histologic features of BK virus seen in 1A.

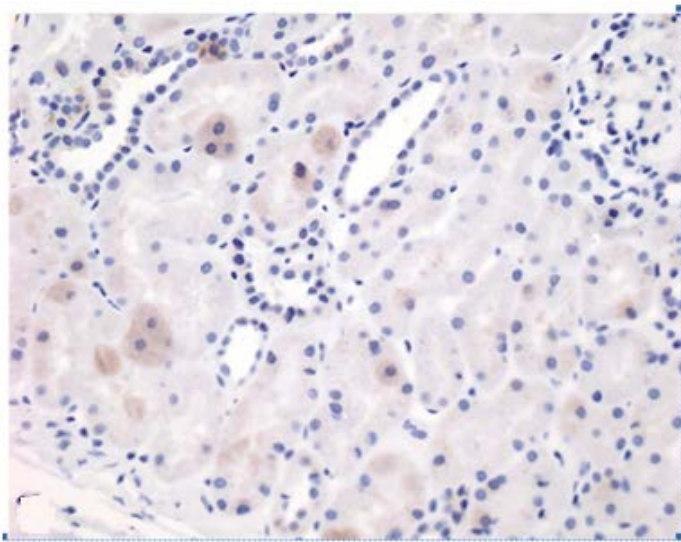


Figure (D): Immunostaining of this case for BK virus shows primarily cytoplasmic reactivity with very questionable nuclear reactivity. A definitive diagnosis of BK virus would likely not be rendered in this case based on these findings; however, BK virus was detected by PCR.

Positive Predictive Value of PCR

To determine the positive predictive value of PCR, we had to reassign our assumption of our “gold standard” assay from H&E with IHC and use PCR as the “gold standard.” After doing this, it was determined that PCR had a positive predictive value of 66% in this series.

Discussion

This study has shown that PCR performed on renal biopsy specimens for the evaluation of BKV is highly specific, but low sensitivity. In patients with strong H&E and IHC evidence of BKV infection, PCR failed to detect the virus in 10 of the 26 cases. This significantly hindered both sensitivity (62%) and positive predictive value (66%) of the assay. We did, however, find that PCR was able to detect BKV in 3 of the 39 “false negative” cases where a clinical suspicion for BKVAN was present (all patients were renal transplant patients) and the virus was not detected by conventional H&E or IHC. Retrospective review of these 3 cases did alert us to weak reactivity of tubular epithelial cells with BKV IHC [Figures C and D]. When

assessing the staining intensity and extent in these three cases, coupled with the lack of definitive histologic evidence, it is unlikely that these cases would have definitively been called “positive for BKV” in a clinical setting. However, the presence of the staining, in our opinion, supports the validity of the PCR results.

As mentioned previously, PCR screening of renal transplant patient’s urine and plasma allows earlier detection of increasing BK viral load [13]. This has led to more proactive management of these patients, reducing the chance where irreversible damage to an allograft takes place before a conventional diagnosis can be made. Renal transplant patients now receive longitudinal followup with urine and plasma PCR for BKV detection [13]. In most cases, urine PCR is used as a primary method of screening, followed by plasma PCR if the viral load exceeded a defined threshold [10, 14]. A BK viral load in the plasma exceeding 10^4 copies/mL has been deemed diagnostic of BKVAN by the American Society of Transplantation and warrants reduction in immunotherapy [15]. In addition, both urine and plasma PCR detection methods have been shown to be highly sensitive and specific for BKVAN [10]. Despite the effectiveness of these PCR methods in urine and plasma, biopsy with IHC is still considered the gold standard of diagnosis and proceeding with this may still be necessitated in some patients with BK viral loads indicative of BKVAN [13].

Despite the well-established clinical utility of PCR screening for BKV, significant concerns have arisen relating to its use. In the study by Chung et al. that described the high sensitivity and specificity of the test, they also found it to have a very lackluster positive predictive value; 55% and 27% for plasma and urine PCR, respectively. Hassan et al. [13] chose to investigate the sensitivity of the designated cutoff of 10^4 copies/mL as advocated by the American Transplantation Society. Of their cases with biopsy-proven BKVAN, 35% (11/35) were found to have plasma BK viral levels below the recommended threshold—yielding a sensitivity of only 65% for the plasma-based PCR [13]. Also of interest was that while 248 patients met the PCR-based criteria for BKVAN, or at least early

BKVAN, based on the American Transplantation Society threshold, only 35 were found to have definitive BKVAN on biopsy. This finding, though, is less troublesome than the false negatives as the test is designed to catch increased viral levels before a significant amount of viropathic damage occurs to tubular epithelial cells.

In addition, problems with the nature of PCR testing itself have been made apparent. There is currently little standardization in protocols that exist for PCR testing and no FDA approved assays are available [13]. This lack of standardization is further complicated by reports that the BKV has numerous subtypes and several polymorphisms have been detected that can significantly alter reliability and reproducibility of PCR testing [1, 16]. With the lack of standardization, different viral variants may test differently on the same platform, altering test sensitivity. Therefore, it is apparent that variable probes used among institutions can lead to discordant results from the same virus [1, 16, 17].

While the clinical utility of PCR as a screening tool for BKV has gained widespread acceptance [11, 12], only few have investigated its role in tissue biopsies. A large study conducted by Schmid et al. [18] examined 302 biopsies from renal transplant patients (only 3 with known BKVAN). BKV was only detected in 8 of the cases by PCR. They reported detection of BKV via IHC in 7 of these cases; 1 case was not available for staining [18]. Costa et al. [19] examined PCR for both BKV and JC viruses (both members of *Polyomaviridae*) in a series of 138 biopsy specimens from kidney and ureter. They detected BKV in 27% of the biopsies; however, they reported their results in genomic equivalents/cell (Geq/cell) and included results as low as 0.3 Geq/cell. Only 2 cases had biopsy-proven BKVAN and viral loads were shown to be over 10^4 Geq/cell [19]. Adeyi et al. undertook laser microdissection of FFPE in 8 renal biopsy specimens and examined them with PCR [20]. Interestingly, they divided their cases in a similar manner as employed in our study; 4 cases had light microscopic evidence of BKVAN, 2 cases were equivocal, and 2 cases were used as controls. They detected BKV in all 4 cases showing BKV on light microscopy,

but did not detect any BKV in the equivocal or control cases [20].

In comparison to these studies, our series, to our knowledge, investigated the largest number of biopsy-proven BKVAN cases. In addition, we had BKV IHC available for all cases, which allowed further comparison of the sensitivity of this well-established modality with PCR. Similar to Schmid et al., we found IHC to be a highly sensitive detection method for BKV in tissue specimens [18]. Furthermore, while the study by Costa et al. [19] showed a high rate of BKV detection in non-BKVAN cases (only 8% of our equivocal cases were positive by PCR), their diagnostic threshold was set very low with the primary objective of quantifying the virus as to correlating it with known BKVAN in tissue specimens, likely accounting for the large discrepancy seen between our results and theirs. The extreme viral load variation in their study further emphasizes the lack of standardization of appropriate viral thresholds in tissue.

As analyzing the reasons of low sensitivity in our study, an obvious difference between ours and others examined is the sample size. In our study, 26 separate cases with histologic and IHC confirmation of BKVAN were examined; only 13 cases combined were reviewed in the other 3 studies discussed [18 - 20]. Besides the much larger sample size used in our study, it is likely that many of the issues and concerns discussed regarding PCR quantitation of BKV in urine and plasma also apply in the tissue specimens. As tissue specimens undergo fixation and paraffin embedding, the quality of the material is likely less so than in fresh urine and plasma specimens. In addition, the much more random nature of a biopsy when compared to samples in the fluid state (i.e., urine and plasma) may also contribute to the lower sensitivity seen in our study. Lastly, the viral particles present in the cases that tested negative may have contained mutations/polymorphisms that allowed them to escape detection with our particular probe set.

In conclusion, we have shown that PCR is a highly specific manner of detecting BKV in renal biopsy specimens. The method seems to significantly lack the sensitivity of H&E with

IHC in most cases, yet it did show value in 3 equivocal cases lacking classic histologic features and minimal reactivity on IHC. Thus, we see the modality of PCR having clinical utility in cases with a high clinical suspicion for BKV where H&E and IHC findings are not conclusive. Our results do not support its use as a primary means of diagnosis or as a replacement for H&E and IHC when a biopsy is performed at the current time. This study also highlights that a much greater understanding of PCR testing for BKV is needed in tissue and a move towards standardization would accelerate the understanding of its use not only in tissue specimens, but increase its current clinical utility in urine and plasma screening for renal transplant patients.

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