Identification of Polyoma BK Virus in Kidney Transplant Recipients by Shell Vial Cell Culture Assay and Urine Cytology

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ABSTRACT

Urine cytology often is used to identify BK virus in kidney transplant recipients in the cytology laboratory. To assess the usefulness of the shell vial cell culture assay to identify BK virus, urine samples from 42 kidney transplant recipients were tested by the urine cytology and shell vial cell culture assays. The shell vial cell culture assay is just as sensitive and specific as urine cytology for the identification of BK virus in kidney transplant recipients.

BK virus (BKV) is a polyomavirus that is ubiquitous among humans. After initial infection in childhood and periods of dormancy, BKV is reactivated in immunocompromised patients.1 It is estimated that 60% to 80% of humans worldwide have antibodies to BK polyomavirus.

Reactivation of BKV occurs in 10% to 60% of renal transplant recipients, and BKV-associated nephropathy (BKVAN) has been found in as many as 8% of kidney transplant recipients.2 Excessive immunosuppression seems to be a predominant factor leading to BKV expression in the kidney graft, sometimes leading to loss of the graft. As a general rule, immunosuppression is reduced in patients with BKV nephropathy.3 The BK viral load decreases in response to the reduction in immunosuppressive therapy.

Identification of BKV in the laboratory includes urine cytology,4 renal allograft biopsy (the “gold standard”),5,6 shell vial cell culture assays,7 polymerase chain reaction (PCR) to detect DNA viral load in the urine and plasma,5 and detection of messenger RNA for the viral VP1 protein in the urine.8 Urine cytology and renal allograft biopsy are the most commonly used laboratory methods to identify BKV. Viral inclusion bodies or “decoy cells” seen in urine cytology assays are characteristic of BKV infection, but the diagnostic sensitivity of decoy cells has been in question for several years because they have been found in patients with normal or stable graft function.9

A shell vial assay was developed for rapid detection of BKV in the clinical virology laboratory. Human fibroblast cells (MRC-5) in shell vials are used to isolate BKV in a few clinical virology laboratories. Because BKV has 70% homology with SV40 (polyomavirus), this assay consists of a cross-reactive monoclonal antibody against epitopes shared by the
T antigen of SV40 and the T antigen of BKV. This method rapidly detects BKV by indirect immunofluorescence.7 Because SV40 infections are confined to monkeys and not commonly present in humans, the use of the cross-reactive monoclonal antibody is not considered an issue. Centrifugation of the shell vials promotes viral infection and allows detection of the virus as early as 24 hours after inoculation.

There is a need for effective, sensitive, rapid, inexpensive, and noninvasive screening methods for identifying patients at risk for developing BKVAN without the use of the invasive renal biopsy and more expensive PCR techniques. By comparing the shell vial assay with urine cytology, we hoped to learn whether the shell vial assay can complement or replace the urine cytology assay for diagnosis of BKVAN.

Materials and Methods

Aliquots of consecutive, random, clean-catch urine specimens from 42 kidney transplant recipients were sonicated to liberate the viral particles and then inoculated into MRC-5 cells shell vials (Diagnostic Hybrids, Athens, OH). After centrifugation at 700g for 45 minutes and overnight incubation at 37°C, a coverslip from each set of shell vials (patient and positive and negative control samples) was stained with the monoclonal antibody to SV40 T antigen at 48, 72, and 96 hours after inoculation. Aliquots of the 42 urine samples also were fixed with a 1:1 dilution with 100% ethanol and centrifuged at 700g for 5 minutes for the cytology assay. The pellets were then diluted with 1 mL of the supernatant and the samples spun in a centrifuge at 400g for 10 minutes. Slides then were prepared and stained with the Papanicolaou stain.

Renal allograft biopsy was performed on 4 patients who had positive urine cytology results and increased plasma creatinine levels. The patients underwent a percutaneous renal biopsy using a standard biopsy gun with an 18-gauge needle. The tissue samples were fixed in formalin and embedded in paraffin. Next, 3- to 4-µm sections were stained with H&E, periodic acid–Schiff, periodic acid–methenamine silver, and Masson trichrome stains. In addition, immunoperoxidase stains for the SV40 large T antigen were performed.

The test results were recorded on an Excel spreadsheet (Microsoft, Redmond, WA) and the 2 methods compared for diagnostic sensitivity and diagnostic specificity. The diagnostic sensitivity was calculated by using the formula TP/(TP + FN) × 100, in which TP represents the true-positive samples in the assay and FN the false-negative samples, or the positive samples that gave a negative result. The diagnostic specificity was calculated by using the formula TN/(TN + FP) × 100, in which TN represents the true-negative samples in the assay and FP the false-positive samples, or the negative samples that give a positive result.

Results

Of the 42 specimens, 4 (10%) were positive by urine cytology and 2 (5%) were positive by shell vial assay ITABLE 11. In the shell vial assay, 1 positive specimen was identified 48 hours after inoculation and the other at 72 hours after inoculation. The apple-green nuclear fluorescence intensified and became brighter with extended incubation time IIMAGE 11. Only 1 of 4 specimens was confirmed as positive by renal biopsy; the others were confirmed as negative for BK-related nephritis. The 2 patients with initially positive urine cytology results and negative shell vial cell culture results were confirmed as positive for the JC virus by molecular technology ITABLE 21.

Compared with the results of the renal allograft biopsy, the diagnostic sensitivity of the shell vial cell culture assay was 100% and the diagnostic specificity was 98%. The urine cytology assay had a diagnostic sensitivity of 100% and a diagnostic specificity of 93% compared with the renal allograft biopsy results ITABLE 31.

ITABLE 11 Results of Urine Cytology and Shell Vial Cell Culture Assay for BK Virus Identification in 42 Specimens

<table>
<thead>
<tr>
<th>Identification Method</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine cytology</td>
<td>4</td>
<td>38</td>
</tr>
<tr>
<td>Shell vial assay</td>
<td>2</td>
<td>40</td>
</tr>
</tbody>
</table>

* Data are given as number of specimens.

IIMAGE 11 Bright apple-green nuclear fluorescence of BK virus in an MRC-5 (human fibroblast cells) shell vial.
Of the 2 patients with positive results for the accepted cytology and shell vial assays, only 1 was confirmed by allograft biopsy as truly positive for BKV. There are a number of reasons that the second patient (case 2, Table 2) had positive results in the cytology and shell vial assays but negative allograft biopsy results. The urine sample for the shell vial assay was obtained before the biopsy was done and, therefore, is not reflective of the patient’s clinical condition at the time of biopsy. Also, even though the allograft biopsy is the gold standard for the detection of reactivated BKV, the biopsy samples only a limited volume of the entire organ. Thus, it may not include tissue with the specific changes diagnostic of BKV infection.

Both assays had similar high diagnostic sensitivities, but the shell vial assay had higher diagnostic specificity than urine cytology for the detection of BKV. Assays with extraordinarily high analytic sensitivity and specificity almost certainly will not perform at these very high levels diagnostically.10 The 2 patients with positive results by urine cytology and negative results by shell vial assay were confirmed as being positive for the JC virus by molecular technology. The shell vial assay will not detect JC virus in the urine sample because JC displays a narrow host range for growth. This is a limitation of the urine cytology assay that identifies decoy cells owing to the presence of one or more polyomaviruses (BK, JC).

It is the current protocol to monitor all kidney transplant recipients after surgery for BKV-related infections. Urine cytology examination is done once every month on every kidney transplant recipient with an elevated serum creatinine level and clinical symptoms consistent with BKV-related infection. Any patient with an initial positive urine cytology result is required to have 2 more consecutive positive results before an allograft biopsy is done to confirm active BKV infection. The BKV status of the 42 patients in this study was monitored. The patients with initial negative urine cytology and shell vial assay results never demonstrated symptoms consistent with BKV-related infection during the study period.

A future study may include the use of shell vial cell culture assay to quantitate BKV load in urine samples obtained from kidney transplant recipients. Quantitation is an important consideration because it has been determined that the BK viral load is related directly to the risk of developing nephritis.11 Quantitative measurement of BKV also is important to monitor the patient’s response to immunosuppression reduction and antiviral therapy.4

Because the current gold standard for detecting BKV is the invasive renal allograft biopsy, there is an urgent need for noninvasive, accurate, and less expensive ways of screening for and monitoring BKV. The PCR technique is reliable, but it is very expensive, and many smaller medical facilities cannot afford to run these technologically advanced tests. The PCR technique also is time-consuming and labor-intensive. Many organ recipients cannot afford to pay for this test because monitoring of the viral load includes periodic testing.

The urine cytology test and the shell vial cell culture assay are inexpensive and available as routine tests in clinical virology laboratories. Our results indicate that the shell vial cell culture assay is more diagnostically accurate than the urine cytology test; the shell vial assay can be used to identify BKV infection in the clinical laboratory.

Cost-effectiveness studies of the urine cytology, shell vial cell culture assay, and PCR techniques should be done to further support the usefulness of these screening assays in identifying BKV in kidney transplant recipients. Medical technologists perform the shell vial assay, whereas the urine cytology assay requires the expertise of trained pathologists, which undoubtedly increases the cost for this assay. In addition, the shell vial cell culture assay is not plagued by false-positive results owing to JC virus infection. Either method will greatly reduce the need for the invasive renal graft biopsy and expensive PCR techniques. Although the shell vial cell culture assay was shown to be just as useful as the urine cytology test commonly used as the screening test to identify BKV, further studies are needed to confirm not only the diagnostic sensitivity and specificity but also the usefulness of this assay in identifying BKV in kidney transplant recipients.

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References