

# Utility of Urinary Cytology for Diagnosing Human Polyoma Virus Infection in Transplant Recipients: A Study of 37 Cases With Electron Microscopic Analysis

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*Human polyoma virus (HPOV) infection is associated with hemorrhagic cystitis, tubulointerstitial nephritis, and renal transplant dysfunction/allograft loss. We evaluated the utility of cytologic examination to detect HPOV infection in 37 urinary cytology (UC) samples (3 bladder washings, and 34 voided samples) from 29 transplant patients, compared to electron microscopic studies (EMS). Evidence of viral infection was found in 11 specimens (30%). Five cases were diagnosed as HPOV by both UC and EMS. One was positive for HPOV by EMS only. Two cases diagnosed as HPOV by UC were demonstrated to be adenovirus (AV) with EMS. Two cases diagnosed as cytomegalovirus (CMV) by EMS had negative UC. One was called HPOV by UC; EMS in this case was negative. Compared to EMS, the sensitivity and specificity of UC for detecting HPOV were 83% and 90%, respectively, with a positive predictive value of 63% and a negative predictive value of 96%. We conclude that UC is a relatively sensitive and specific method for detecting active HPOV infection in transplant patients, and is important in light of the clinical significance of HPOV infection in transplant recipients. The sensitivity and*

*accuracy of UC for diagnosing HPOV can be increased by adding EMS. Diagn. Cytopathol. 2001;25:376–381.*

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Since it was first identified in the urine of a renal transplant recipient in 1971, human polyoma virus (HPOV, or BK virus, after the initials of the patient from whom it was isolated) has been linked with urologic pathology.<sup>1</sup> The virus belongs to the family Papovaviridae and is nonenveloped, with circular, double-stranded DNA.<sup>2</sup> Experts postulate that individuals are first infected by HPOV in early childhood by inhalation of viral particles. Immunocompetent individuals are able to control active infection, but viral particles have been shown to persist in urothelial cells in a latent state.<sup>2,3</sup> It is well-known in cytology that HPOV-infected urothelial cells can mimic the features of bladder carcinoma on light microscopy (“decoy” cells).<sup>4</sup> However, it is less well-known that HPOV infection itself can be clinically significant, being associated with ureteral stenosis, hemorrhagic cystitis, and tubulointerstitial nephritis. In addition, HPOV reactivation in renal transplant recipients can lead to severe allograft dysfunction or even loss of the organ in severe cases.<sup>2–7</sup>

Given this background, we assessed the utility of urinary cytology (UC) for correctly detecting and monitoring active infection by HPOV in allograft recipients. Specifically, we sought to determine the sensitivity and specificity of UC for diagnosing HPOV compared to electron microscopic studies (EMS) in urine samples. These results were correlated

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**Table I.** Main Disease That Led to Transplant and Associated Conditions Found in Our Patients (Total of 29)<sup>a</sup>

<i>Main disease</i>	<i>N</i>	<i>Associated conditions</i>	<i>N</i>
<b>Renal transplant patients</b>			
Diabetes mellitus	9	Hypertension	3
		Chronic lymphocytic leukemia	1
		Uterine adenocarcinoma	1
Primary glomerulopathy	8	Hypertension	1
		Vasculitis	1
Systemic lupus erythematosus	3	Diabetes mellitus	1
End-stage renal disease of unknown cause	1		0
Hypercholesterolemia	1		0
Obstructive nephropathy	1		0
Reflux nephropathy	1		0
Renal artery stenosis	1		0
Severe atherosclerosis	1		0
<b>Bone marrow transplant patients</b>			
Acute lymphocytic leukemia	1	Graft vs. host disease	1
Large-cell non-Hodgkin's lymphoma	1		0
<b>Liver transplant patients</b>			
Alcoholic cirrhosis	1		0

<sup>a</sup>N, number of patients.

with clinical data and compared to diagnoses rendered on renal tissue biopsies, when available.

## Materials and Methods

Thirty-seven urine samples from 29 transplant patients were submitted to the Cytology Laboratory at the University of Wisconsin-Madison over a 2-yr period for evaluation for HPOV infection. The samples averaged 10 ml in volume, and were split into two 5-ml aliquots for light microscopy and EMS. The samples included 3 bladder washings and 34 voided urine specimens. Clinical information from all patients was evaluated for age, sex, medical diagnosis necessitating transplantation, associated medical conditions, and clinical symptoms prompting UC. Each UC specimen was evaluated by a cytopathologist, who rendered a final diagnosis without knowledge of the corresponding EMS result.

The aliquot designated for cytologic analysis was centrifuged at 1,500 rpm for 10 min. Two slides were then prepared from cytocentrifuge material, fixed in 95% ethanol, and stained via the Papanicolaou technique. The second aliquot was also concentrated by centrifugation, and the sediment was fixed in glutaraldehyde. The specimen was then recentrifuged, embedded in 4% Nobles agar, postfixed in Caulfield's osmium tetroxide, dehydrated in series of graded alcohol, and reembedded in Epon-Spurr. Ultrathin sections (900 Å) were examined with a Hitachi H-600 electron microscope (San Jose, CA) by two pathologists with extensive experience in electron microscopy. EMS results were also reported without knowledge of the corresponding UC diagnosis.

In addition, renal biopsy tissue was obtained as part of the evaluation in 15 cases. These were fixed in 10% buffered formaldehyde and embedded in glycol methacrylate. Thin sections were cut for each case (1.5 µm) and stained with

hematoxylin-eosin (H&E). These slides were evaluated by two pathologists with expertise in the practice of renal pathology. Diagnoses on this material were rendered without knowledge of the UC results.

## Results

The 29 patients ranged in age from 9–67 yr (mean, 43 yr), and included 12 females and 17 males. Twenty-six of these individuals were renal allograft recipients. In addition, 2 patients had undergone allogeneic bone marrow transplantation, and 1 had received an orthotopic liver transplant. The main disease that led to the transplant and the associated conditions for each patient are given in Table I.

Medical indications prompting evaluation for HPOV included: 1) abnormally elevated serum creatinine levels (81%); 2) hematuria (7%); 3) graft tenderness (6%); 4) pyuria (3%); and 5) fever of unknown origin (3%).

Overall, evidence of viral infection was found in 11 of 37 specimens (30%). These results are summarized in Table II. Specifically, 5 cases were diagnosed as HPOV by both UC and EM (Fig. 1). Two cases diagnosed as HPOV by UC were demonstrated to be adenovirus (AV) by EMS (Fig. 2). One specimen with negative UC was positive for HPOV by EMS. Also, 2 cases diagnosed as cytomegalovirus (CMV) by EMS had negative UC. Finally, 1 case with negative EMS was called HPOV by UC.

Compared to EMS, the sensitivity of UC for detecting HPOV was 83%, with a specificity of 90%. The positive predictive value for UC for HPOV infection was 63%, and the negative predictive value was 96%.

Viral cytopathic changes were detected in only 2 of 15 (13%) renal-tissue biopsies (Table III). One was diagnosed as HPOV, while the other was diagnosed as CMV (Fig. 3). These light microscopic diagnoses were confirmed by EMS.

**Table II.** Viral Detection: UC Compared to EMS (Total, 37 Cases)

Urinary cytology	Electron microscopy			Total
	Positive	Negative	Insufficient no. of cells for EMS	
Positive	7 <sup>a</sup>	1 <sup>c</sup>	0	8
Negative	3 <sup>b</sup>	25	1	29
Total	10	26	1	37

<sup>a</sup>Seven cases had positive UC and EMS. Five cases were diagnosed as human polyoma virus by both UC and EMS. Two cases diagnosed as human polyoma virus by UC were demonstrated to be adenovirus with EMS.

<sup>b</sup>Three cases had negative UC and positive EMS. EMS demonstrated cytomegalovirus in 2 cases and human polyoma virus in 1 case.

<sup>c</sup>One case was called human polyoma virus by UC, and no viral particles were identified by EMS.

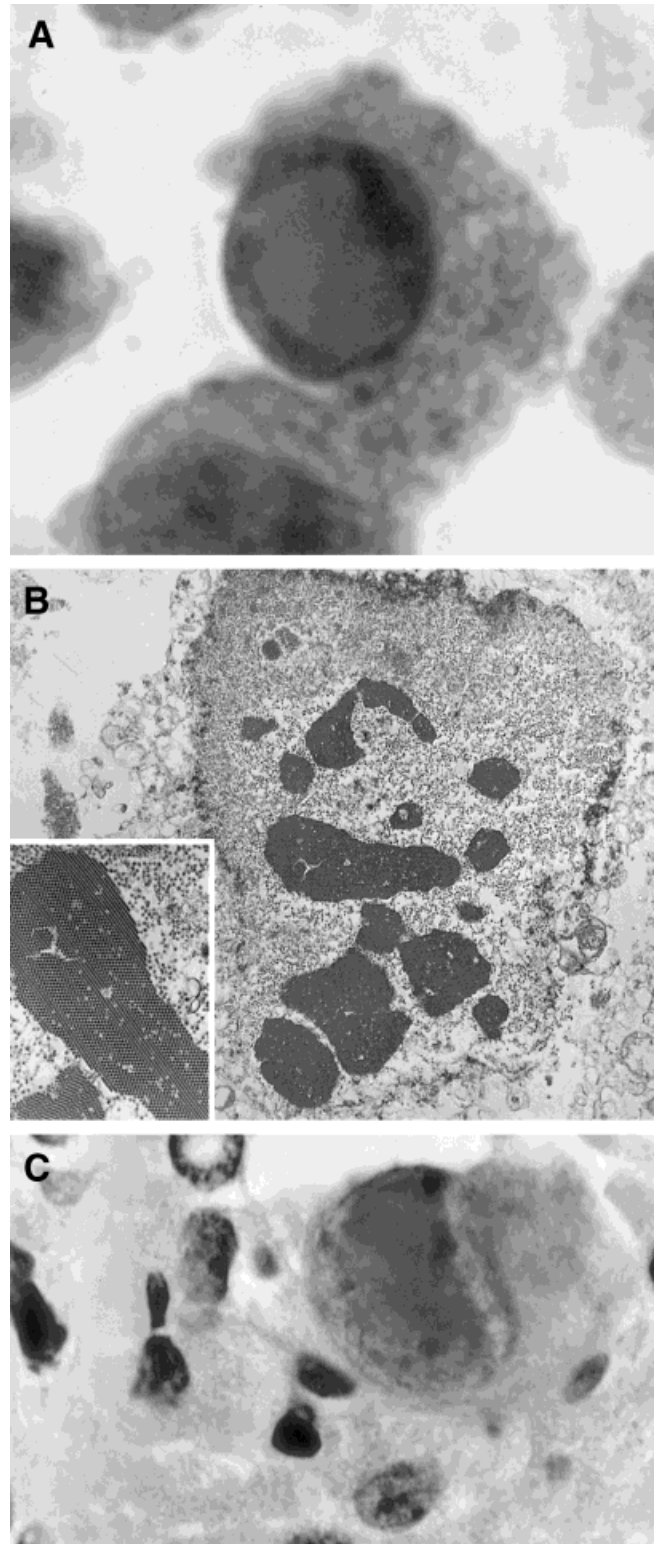
One case with no cytopathic effect on tissue had positive evidence of HPOV viral infection by UC and EMS.

Overall, unequivocal evidence of active HPOV infection was found in 6 specimens (16%), including 1 detected only by EMS. All of these samples came from 3 adult renal transplant patients (RTP). They included 1 sample from a female patient with systemic lupus erythematosus, 4 from a male patient with end-stage nephropathy secondary to diabetes, and 1 from a male patient with chronic glomerulonephritis of undetermined etiology. The samples containing AV and CMV belonged to adult diabetic RTP.

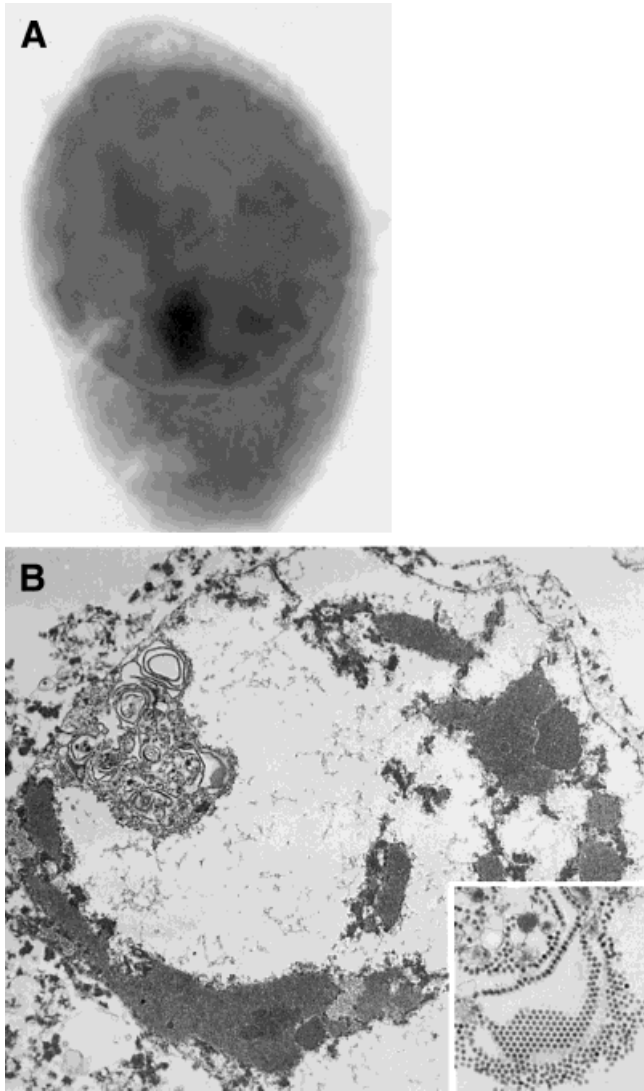
## Discussion

BKHPDV infection has worldwide distribution.<sup>2</sup> This virus has been shown to infect transitional epithelium, renal tubular epithelium, and the parietal epithelial layer of Bowman's capsule.<sup>8</sup> In patients who are immunosuppressed, reactivation of HPOV is accompanied by shedding of virus-infected urothelial cells, and has been associated with significant urologic pathology.<sup>9</sup>

The presence of HPOV is now considered an extremely important finding in immunocompromised individuals and, particularly, in renal and bone marrow transplant allograft patients.<sup>2,3</sup> Polyoma virus infection in renal transplant recipients was first described in the early 1970s.<sup>10</sup> However, little attention was focused on this infection until recently. Because polyoma virus is associated with a chronic interstitial infiltrate that mimics rejection (see below), the diagnosis may have been underappreciated. Certainly, the use of more potent immunosuppressive regimens with mycophenolate mofetil or tacrolimus may be leading to an increased incidence of infection.<sup>11</sup> In any event, the clinical implications of infection with HPOV virus in RTP are significant and can induce severe nephropathy. It was found that tubular necrosis is an important cause of graft dysfunction in renal allograft patients, and that it is a consequence of BK virus replication.<sup>12</sup> In one recent series, 67% of grafts were lost in kidney transplant recipients who received therapy for acute rejection.<sup>6</sup> In the same series, a reduction of immu-



**Fig. 1.** Human polyoma virus (HPOV). **A:** Urinary cytology of HPOV-infected urothelial cell (Papanicolaou stain,  $\times 1,000$ ). Note similarity to Figure 2A. **B:** Ultrastructure of HPOV-infected urothelial cell (electron micrograph printed at  $\times 16,000$ ). **Inset:** Higher magnification, displaying small round viral particles (electron micrograph printed at  $\times 30,000$ ). **C:** Tissue section, demonstrating cytopathic effect in renal tubular epithelial cells (H&E,  $\times 1,000$ )



**Fig. 2.** Adenovirus (AV). **A:** Urinary cytology of AV-infected urothelial cell ("smudge cell") (Papanicolaou stain,  $\times 1,000$ ). Note similarity to Figure 1A. **B:** Ultrastructure of AV in a urothelial cell (electron micrograph printed at  $\times 15,000$ ). **Inset:** Higher magnification, showing unique structure of AV (electron micrograph printed at  $\times 64,000$ ).

nosuppression resulted in continued, but impaired, graft function. As yet, there is no effective antiviral therapy for HPOV. In recipients of bone marrow transplants, infection with HPOV is associated with hematuria and hemorrhagic cystitis.<sup>2,3</sup>

Histologically, it is very difficult to differentiate BK nephritis from acute rejection, because both show tubulitis.<sup>12</sup> We actually may have a better chance to detect HPOV by using UC. The failure of histologic sections to reliably distinguish acute rejection from HPOV nephropathy in some cases can be crucial because of contrasting therapies. Acute rejection is treated by increasing immunosuppression, and BK nephropathy is generally treated by decreasing the dose of immunosuppressive drugs. An alternative diag-

nostic modality is called for. A noninvasive and sensitive diagnostic tool such as UC<sup>12</sup> could be seen as an extremely important aid in the management of these difficult patients. In addition, when monitoring renal allograft patients, if viral cytopathic effect is seen in UC specimens associated with an inflammatory sediment, renal biopsy is indicated.<sup>12</sup>

Many authors affirm that the cytologic appearance of HPOV is characteristic enough that infection can be diagnosed solely by UC.<sup>13</sup> Our study indicates that the cytopathic changes seen in UC, generally attributed to HPOV, are not entirely specific and appear similar to those changes seen and described in adenovirus-infected cells<sup>14</sup> and sporadically in degenerated cells.

We found UC specimens containing urothelial cells displaying enlarged hyperchromatic nuclei of variable density, with no visible halo or nucleoli and scant, partially degenerated cytoplasm. EMS demonstrated AV in some of these cases and HPOV in others. Therefore, the presence of those cytopathic changes consistent with so-called "smudge cells"<sup>14</sup> should be evaluated considering the clinical setting, and viral identification studies may be performed in selected cases.

Definite identification of HPOV infection is done by conventional cell culture and viral isolation, indirect immunofluorescence, dot enzyme immunoassay, and DNA-DNA hybridization assay.<sup>12</sup> Recently, extremely sensitive molecular tests such as the polymerase chain reaction (PCR), that can identify latent viral infections that cause clinical disease only infrequently, have evolved toward semiquantitative tests to monitor viral load.<sup>2</sup> These techniques have the potential to be performed in urine and may offer an excellent future alternative for the detection of HPOV nephritis.<sup>2</sup> However, these tests are not usually available, and cost-effectiveness needs to be assessed. As a clinically practical and valuable option, EMS can confirm the presence of viral particles, such as HPOV, CMV, and adenovirus.<sup>11</sup> Additionally, JC virus (the etiologic agent of progressive multifocal leukoencephalopathy, an HPOV with a similar morphology to BK virus) has been associated with genitourinary pathology.<sup>1,2</sup> Therefore, in selected cases, cultures or molecular studies should be performed for definitive identification.<sup>15</sup>

Immunostains for HPOV and AV are also available, but the experience with and application to UC are still limited. However, they can potentially identify the virus type and be an interesting alternative to EMS.

Only rarely has HPOV infection been reported in recipients of solid organ grafts other than kidney.<sup>8</sup> Maintenance of immunosuppression in these patients often equals or exceeds that used for RTP, suggesting that additional factors other than level of immunosuppression play a role in the reactivation and pathogenesis of HPOV nephritis.<sup>8</sup> The only liver transplant patient who was part of our study had negative UC and EMS; no renal biopsy was obtained.

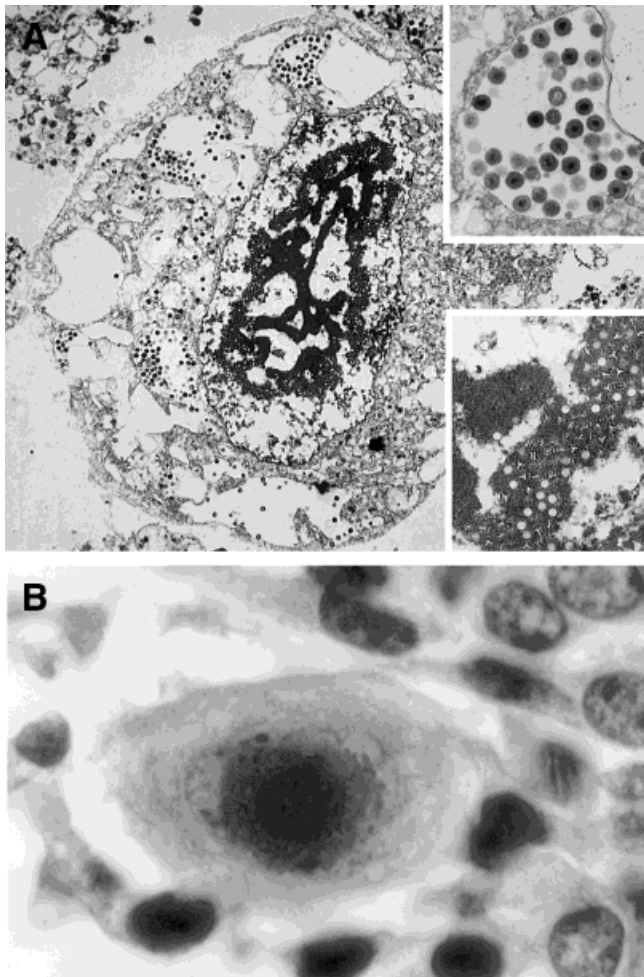
**Table III.** Viral Detection: Tissue Biopsies Compared to UC and EMS (Total, 15)

Renal BX	Positive UC			Negative UC			Total
	+EMS	-EMS	Insuff. <sup>a</sup>	+EMS	-EMS	Insuff.	
Positive	1 <sup>b</sup>	0	0	1 <sup>c</sup>	0	0	2
Negative	1 <sup>b</sup>	0	0	0	11	1	13
Total	2	0	0	1	11	1	15

<sup>a</sup>Insuff., insufficient number of cells for EMS.

<sup>b</sup>EMS revealed HPOV.

<sup>c</sup>EMS revealed CMV.



**Fig. 3.** Cytomegalovirus (CMV). **A:** Ultrastructure of CMV particles in a urothelial cell (electron micrograph printed at  $\times 10,200$ ). **Upper inset:** Cytoplasmic virions. **Lower inset:** Nuclear virions (both printed at  $\times 38,000$ ). **B:** Tissue section, showing characteristic cytopathic effect of CMV in renal tubular epithelial cells (H&E,  $\times 1,000$ ).

A review study reported viral cytopathic changes of HPOV in renal graft tissue in 7 of 13 (54%) patients with positive UC when the whole allograft kidney was evaluated (obtained by nephrectomy or autopsy).<sup>9</sup> Only in 1 of our 2 cases who had HPOV in UC and concurrent kidney biopsy, were HPOV cytopathic changes detected in renal tissue sections. This case also had positive confirmatory EMS. Therefore, negative biopsies with positive UC and EMS can

be expected, particularly considering that biopsy material was evaluated instead of nephrectomy specimens. The urothelial cells with cytopathic changes of HPOV seen in our tissue biopsy material and UC appeared similar, and were readily identified by their large-sized nuclei that contained dense basophilic homogeneous inclusion bodies, and by their partially degenerated cytoplasm.

It is standard teaching in cytopathology that the cytopathic changes of HPOV-infected urothelial cells can mimic the features of bladder carcinoma in UC.<sup>4</sup> Similar to malignant cells, infected nuclei are enlarged and hyperchromatic. However, the infected cells often display a central, relatively large, homogeneous (“smudgy”) area of dense chromatin, plus a small rim of clumped nuclear material<sup>4</sup> instead of the coarse chromatin granules and chromatin clearing of malignant urothelial cells. Also, HPOV-infected cells are generally found singly and with eccentrically located nuclei.<sup>4</sup> Thus the dyscohesion of malignant processes is mimicked. Carcinoma cells tend to form disorganized tissue fragments displaying cellular crowding and nuclear overlapping.

In one of our cases, HPOV was detected by EMS but not by UC. In our defense, our samples did exhibit extensive cell degeneration. In addition, partial cytolysis and cell degeneration are probably not as important as the total viral load for EMS. If the urine sample contains at least a million viral particles per milliliter, EMS should be able to identify viral particles.<sup>16</sup> Another case was positive for HPOV on UC and EMS, but no cytopathic changes were seen in tissue sections, which showed extensive tubulitis, inflammation, interstitial fibrosis, and arteriolo-nephrosclerosis. Additionally, this case was diagnosed as acute rejection of renal allograft. The patient underwent a second kidney transplant, 9 days after being diagnosed as positive for HPOV by the UC sample. We do not know how different the management would have been, if tissue viral changes had been seen. Possible explanations for not detecting HPOV histologically are either the severe cytolysis/inflammation caused by rejection and/or the infection obscuring the cytopathic effect, or biopsy sampling error.

The use of UC in the hunt for the BK virus now seems to be more than a morphologic curiosity. The infection can have important clinical consequences and potentially ruin all the physical, emotional, and financial effort that goes

into a transplant. In conclusion, UC is a safe, noninvasive, and reliable diagnostic tool for identifying viral cytopathic effects in urothelial cells, and deserves more widespread use in the monitoring of RTP. In the appropriate clinical and laboratory setting, there is therapeutic significance to a positive UC diagnosis. Our findings also indicate that the sensitivity and accuracy of UC are increased when combined with EMS. For these reasons, we suggest that part of the UC sample be simultaneously used for EMS, or at least be saved and stored in EM fixative when HPOV is clinically suspected.

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