Polyomavirus Infection of the Urinary Tract Presenting as Hemorrhagic Cystitis in an Immunocompetent Five-Year-Old Boy

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Human polyomavirus infection, which can be detected morphologically on Pap-stained routine urine cytology specimens, is most commonly encountered in immunocompromised patients and is a well-described complication of renal transplantation. We present a case of an immunocompetent 5-year-old boy with a sudden onset of dysuria and hematuria due to a self-limited polyomavirus urinary tract infection detected on routine urine cytology and confirmed with real-time PCR. Although rare cases of nonhemorrhagic cystitis have been reported, to the best of our knowledge this is the first case of hemorrhagic cystitis occurring in this setting. Diagn. Cytopathol. 2008;36:375–378. © 2008 Wiley-Liss, Inc.

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Human polyomavirus infections most often occur in immunosuppressed patients. Rare examples of symptomatic infections in young immunocompetent hosts have been described, typically manifesting as nonhemorrhagic cystitis. We present a case of self-limited hemorrhagic cystitis in a 5-year-old boy due to polyomavirus infection that was confirmed with real-time PCR.

Case Report
A previously healthy 5-year-old boy presented to a community hospital with the sudden onset of dysuria followed by gross hematuria. His initial evaluation revealed many atypical epithelial cells in the urinary sediment that were concerning for a neoplastic process. He was referred to a regional medical center for further evaluation 3 days after the onset of symptoms. He was not taking any medications and had no prior history of symptoms related to the urinary tract. There was no family history of immune system-related disease. The external genitalia were normal. An abdominal ultrasound revealed normal kidneys without evidence of hydronephrosis. A post void residual volume of 190–200 ml was noted on urinary bladder ultrasound.

The initial Pap-stained urine cytology exhibited numerous virally-transformed urothelial cells characterized by large intranuclear homogeneous basophilic inclusions with some peripheral clumping of chromatin on the nuclear membrane (Figs. C-1 and C-2) associated with many degenerated cytologically atypical “decoy” cells with hyperchromatic nuclei (Fig. C-3). Repeat urine samples over a 14-day-period showed rapidly decreasing numbers of virally-transformed cells with an eventual return to negative urines on routine cytology preparations, correlating with the resolution of the urinary symptoms.

Cystoscopic evaluation of the bladder performed after the resolution of symptoms revealed a normal bladder and urethra, without evidence of masses or calculi, and unobstructed ureteral orifices with clear urinary efflux.

Methods
The initial urine samples were received fresh, and then combined with an equal volume of 50% ethanol. After
centrifugation, smears were prepared from the urinary sediment and Pap-stained. The subsequent urine samples were received in CytoRich Blue and prepared for cyto logic evaluation using the liquid-based SurePath™ method.

A cell block obtained from the sediment of the initial urine collection was tested for polyomavirus DNA using real-time PCR for BKV and JCV. Real-time PCR was performed using the primers described by Beck et al., with SYBR Green (ABI, Foster City, CA) detection. A post-PCR melt was done to differentiate the BKV from JCV amplicons. An internal control gene (Factor V) was also amplified to determine if the extracted DNA sample was adequate for amplification. Briefly, extracted DNA was added to a master mix containing 1X SYBR Green Master Mix (ABI, Foster City, CA) and 1 μM forward and reverse primers. The primer sequences were: BKJC forward - 5’TCTCTAGTAGCAAGGGATGC; Factor V forward- 5’GCTGCCCATGAATAGCAG; Factor V reverse- 5’CTACTTCTAATCTGTAAGGCAG. Cycling was performed in the Smart Cycler II (Cepheid, Sunnyvale, CA) using the following conditions: 95°C, 10 minute; 40 cycles of 95°C for 15 seconds, 60°C for 30 seconds (optics on), 72°C for 30 seconds. Melt curve analysis has become a routine method for the analysis of sequence variants using common real-time PCR platforms. Based on the melting temperature or $T_m$ of a specific amplicon, the melt curve identifies a characteristic $T_m$ that can distinguish between amplicons that differ by only a single base. In this study, a melt curve analysis was performed using the SYBR Green channel where melt data were collected from 40 to 95°C at a ramping rate of 0.2°C/seconds with the optics on. The results of this analysis demonstrated the presence of viral DNA sequences in the patient specimen that had $T_m$s equivalent to the corresponding BKV positive...
control (Fig. C-4), thus confirming the presence of BK polyomavirus infection.

**Discussion**

Human polyomavirus, a genus of papovaviruses, has two known types, JCV and BKV, both of which can result in urinary tract infections. Typically these infections occur in immunocompromised hosts and may commonly complicate renal transplantation.²

The intranuclear inclusions of polyomavirus infection can be detected in routine Papanicolaou-stained urine cytology preparations. The infected cells have characteristically large, dense, homogeneous basophilic nuclear inclusions with some peripheral clumping of chromatin on the nuclear membranes (Figs. C-1 and C-2).³ Polyomavirus infection induces marked cellular degeneration and is typically associated with degenerated urothelial cells that have atypical hyperchromatic nuclei which can be erroneously interpreted as carcinoma (Fig. C-3). These latter cells are known as “decoy” cells, a term coined by Andrew Ricci, a cytotechnologist working in concert with Leopold Koss at the Memorial-Sloan-Kettering Cancer Center in the 1950s. The term underscores the benign nature, yet atypical cytomorphologic appearance of these degenerated cells.⁴ The association of decoy cells with polyomavirus infection was subsequently established in the early 1970s. The presence of many decoy cells in a urine sample should be noted since the finding may herald polyomavirus infection, especially in immunosuppressed patients.

Given the marked degenerative changes induced by polyomavirus, there are times when the cytomorphologic features are equivocal and confirmation of the viral infection with adjuvant studies is prudent. Both electron microscopy and PCR are established methods⁵–⁷ to confirm polyomavirus infection. More recently, real-time PCR has emerged as a powerful and preferred tool for the detection and typing of a variety of viruses. The real-time PCR technique has several advantages over the traditional PCR. Some of these include its more rapid turn-around time due to the elimination of separate post-amplification analysis steps and increased sensitivity and specificity due to novel detection methods. We confirmed the presence of the BK polyomavirus using this methodology (Fig. C-4).¹

Seroprevalence studies have shown that most primary infections with BKV occur in the first decade of life, while infections with JCV are more prevalent in children greater than 10 years of age.³ Little is known about the primary source of human exposure or the means of viral transmission. Although there is evidence that children can be infected at an early age,⁸ it is unusual for them to develop clinically evident hemorrhagic cystitis without immunosuppression. Rare cases of nonhemorrhagic cystitis have been reported in immunocompetent children at 3–5 years of age.⁹¹⁰ In one well-documented case reported in 1988, a 4-year-old boy with a history of repeated urinary tract infections presented with dysuria and urothelial viral inclusions in a routine urine sample confirmed by electron microscopy as polyomavirus. The patient’s urinary symptoms subsided spontaneously, and he had no further manifestations of the viral infection at the time of publication.¹⁰ The case that we report represents another rare example of a self-limited symptomatic urinary tract polyomavirus infection (BKV) in a young immunocompetent host, but one that manifested as hemorrhagic cystitis. We apparently witnessed its evolution from a high viral load associated with dysuria and hematuria to eventually negative urines on routine cytology preparations and a resolution of symptoms within 14 days.

**References**

1. Beck RC, Kohn DJ, Tuohy MJ, Prayson RA, Yen-Lieberman B, Procop GW. Detection of polyoma virus in brain tissue of...


