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Diagnostic utility of urine cytology in early detection of polyomavirus in transplant patients

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Introduction Polyomavirus-associated nephropathy (PVAN) is one of the most common disease affecting transplant patients, mainly caused by BK polyomavirus (BKV) and with <5% of the cases caused by JC polyomavirus (JCV). Screening and early intervention, including appropriate reduction in immunosuppressive therapy, are critical to reduce allograft loss. The presence of decoy cells in the urine is a characteristic cytopathic effect of polyomavirus. The goal of this study was to investigate the significance of decoy cells in urine cytology in transplant patients, comparing with the plasma viral replication level detected by the real-time quantitative BK virus polymerase chain reaction test (Qt-BK PCR).

Methods A cohort of post-transplantation patients with serum BKV level monitored by Qt-BK PCR from 2008 to 2013 was studied. Among them, 35 patients had both urine cytology (UC) analysis and Qt-BK PCR performed. The clinical presentation along with the available UC slides were retrieved and reviewed.

Results Compared with Qt-BK PCR, the sensitivity, specificity, positive predictive value, and negative predictive value of urine cytology analyzed within one week apart were 92%, 71%, 85%, and 83%, respectively. The accuracy of the UC was 84%. More interestingly, UC played a key role in identifying a case of JCV associated PVAN whereas Qt-BK PCR from both urine and plasma failed to detect this virus.

Conclusion Our data suggests that urine cytology is a sensitive surveillance test for early detection of polyomavirus in transplant patients, and it is particularly useful to screen for rare JC polyomavirus.

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Introduction

BK polyomavirus (BKV) was first isolated from the urine of a kidney transplant recipient in 1971 at the Central Public Health Laboratory in London.[1] However, the role and significance of BKV in kidney transplantation were not clear until many years later when polyomavirus-associated nephropathy (PVAN) in kidney transplantation was defined and recognized as one of the leading causes for allograft failure.[2,3] The estimated incidence of PVAN in kidney transplant recipients was reported ranging from 1% to 10% with an overall graft failure rate of 15% to 80% of cases within 5 years.[4] PVAN is primarily caused by BKV and <5% of the cases have been attributed to JC polyomavirus (JCV).[5-7] BKV and JCV are members of the polyomaviridae family and are ubiquitous in the general population. These viruses remain latent following primary infection, especially in the reno-urinary tract. Reactivation of these viruses plays a major role in developing PVAN. In most instances, PVAN typically occurs at a mean period of 1 year post-transplantation but may occur as early as 6 days or as late as 5 years. The clinical presentation may be insidious and nonspecific, with varying degrees of renal dysfunction and elevated serum creatinine levels. Currently, there is no safe and effective antiviral therapy for PVAN and the only documented effective treatment is reduction of the immunosuppressant therapy.[8] Therefore, early detection of viral reactivation and replication before the development of PVAN is necessary to prevent subsequent kidney allograft failure.

Methods for monitoring BKV infection in kidney transplant patients include: urine cytology (UC) with cytologic detection of decoy cells, urine and plasma quantitative-BK polymerase chain reaction assays (Qt-BK PCR), renal biopsy with ancillary studies such as electronic microscopy examination for viral aggregates, SV-40 immunohistochemical staining, and fluorescent in situ hybridization.[9] Detecting urinary decoy cells in UC specimens has been recommended as a reliable and cost-effective surveillance test.[10,11] The drawbacks of UC are the presence of nonspecific viral cytopathic changes, cellular degeneration, and morphologic mimic of high-grade urothelial carcinoma cells, which may lead to misinterpretation of polyoma cytopathic effect. Qt-BK PCR is the method of choice for screening and monitoring BKV infection. The current commercially available kit does not detect JC virus, however. Renal transplant biopsy is the "gold standard" to make the definitive diagnosis of PVAN, although a negative biopsy result cannot rule out a PVAN diagnosis because of to the sporadic distribution of the viruses. The aim of this study was to compare UC with plasma Qt-BK PCR assay to determine the diagnostic utility of UC as a screening/monitoring tool for polyomavirus infection in transplant patients.

Materials and methods

The study was approved by the institutional review board at Cleveland Clinic, and the necessity of informed consent was waived for this retrospective study. A cohort of post-transplantation patients who were monitored by Qt-RT PCR from 2008 to 2013 was studied. The medical records of these patients were reviewed for demographic data, clinical presentation, pathologic diagnoses, and outcome.

Urine cytology

For each transplant patient in the cohort, the pathological report database (CoPath-Plus) was searched to identify the list of patients who had a urine cytology sample sent for analysis. Because of the nature of retrospective study, the patients with both UC and Qt-BK PCR tested within 5 weeks were included in the study. Qt-BK PCR testing performed within one week of UC was defined as co-testing. The urine cytology slides were retrieved, and reviewed for diagnostic confirmation. The sensitivity, specificity, positive and negative predictive values (PPV and NPV), and accuracy of the urine cytology were calculated and compared with plasma Qt-BK PCR.

Real-time quantitative BK and JC polyomavirus polymerase chain reaction test (Qt-BK PCR)

The DNA from plasma specimens from these patients were extracted using the MDX instrument with the QIAamp Virus BioRobot MDx Kit (Qiagen, Germantown, Md.; catalog #965652). The Qt-BK PCR assays were performed at molecular laboratory of Cleveland Clinic using the Artus BK Virus RG PCR ASR kit (Qiagen). The test results were reported as detected versus nondetected; for specimens with BKV detected, a viral load within the range from 500 to 5,000,000 copies/mL was reported. A LightCycler PCR assay, which has been previously described, was used to differentiate the BK virus from JC virus using melt curve analysis.[12-14]

Results

UC is compatible with Qt-RT-PCR test

Thirty five (35) patients were identified who had both urine cytology analysis and Qt-BK PCR tests performed. Six of these patients had these two tests performed more than 5 weeks apart and were excluded from the study. Two patients who had a urine PCR test instead of a plasma Qt-BK PCR test were also excluded from the study. Among those 27 patients who met our inclusion criteria, 20 were men and 7 were women, with age ranging from 26 to 77 years old (mean: 53.4 years). Twenty-three (23) patients had kidney transplantation, 3 patients had bone marrow/stem cell transplantation, and 1 patient had lung transplantation. The mean age at transplant was 44 years old, ranging from 21 to 75 years old. A total of 32 UC specimens were identified in