Monitoring BK Virus in Kidney Transplant Patients

Tiffany Page, Diasorin
In collaboration with Dr Neisha Jeoffreys, Westmead Hospital

A new, benchtop molecular analyser allows renal transplant centres to monitor urine and serum BK viral loads in house, permitting earlier diagnosis and management of BK virus associated nephropathy (BKVAN) in renal transplant recipients.

BK Virus Infection

Named after the renal transplant patient (with initials B.K.) that it was first isolated from in 1971, BK virus (BKV) is a Polyomavirus, characterised by its nonenveloped, icosahedral capsid and its circular, double-stranded DNA genome. Although BKV is prevalent around the world, estimated to have infected more than 80% of the global population, infection with the virus is usually asymptomatic or associated with only mild respiratory tract symptoms in healthy individuals [1]. Following primary infection, which typically occurs in early childhood, the virus persists in a latent form in the kidneys and urinary tract of its host [2].

Reactivation of BKV can occur in immunocompromised and immunosuppressed individuals [3]. In most cases reactivation of the virus is benign but it can pose a particular challenge for renal transplant patients. In such cases immunosuppression can cause a lytic BKV infection that results in viruria in 30-50% and viremia in 13-22% of renal transplant recipients [4]. BKV infection is one of the most common viral complications to affect renal allografts [5]. It can lead to BKV associated nephropathy (BKVAN) in up to 10% of renal transplant recipients, and is associated with graft failure in 15-80% of affected patients [3, 6-9].

Not every latent infection leads to viral reactivation and BKVAN in renal transplant patients. In addition to immunosuppression, other risk factors, such as intragraft inflammation and host-specific immunity have been suggested [3]. The progression of BKVAN may occur without obvious signs or symptoms, other than raised serum creatinine, and so it is often misdiagnosed [9].

Management of BKVAN

Treatment of BKV infection and BKVAN in renal transplant patients usually involves a reduction or modification of immunosuppressive therapy. It is generally agreed that early diagnosis and treatment are extremely important to prevent damage to the allograft [3, 9]. At a later stage of infection, when intragraft inflammation has developed, reduction of immunosuppression may not be effective and may even be detrimental to the allograft [3]. For this reason, frequent monitoring of BKV in renal transplant patients, to detect early onset BKV infection, is recommended to ensure timely intervention [3].

Confirmation of BKVAN is performed by histological examination of an allograft biopsy sample. However, clinical intervention is often based on the presence of viral replication as a surrogate marker and early indicator of BKV infection. For this reason, non-invasive urine and blood tests have value in screening for BKV reactivation, monitoring the clinical course of infection, or monitoring response to therapy [3].

Urine cytology has been used to screen for BKV reactivation in renal transplant recipients. However, since the virus may be shed in the urine of healthy individuals, quantitative results are required for this method to have diagnostic value [3]. Furthermore, accurate interpretation of cytology results requires training and expertise as it is often difficult to distinguish BKV from other viral infections [9].

Recently, molecular techniques for the detection and quantification of BKV in blood and urine have become available. Such methods offer greater specificity for BKV and provide a valuable tool for identifying patients at risk of BKVAN before renal function deteriorates.

Monitoring BK Viral Loads

Quantitative measurements of BK viral load in urine and blood by molecular techniques are useful for monitoring the course of BKV infection [9] and for predicting the development of BKVAN [4, 7, 10, 11]. Viral reactivation can be detected in the urine several weeks before the virus is detected in the blood, and viremia can be detected months before histological evidence of BKVAN is present [3]. Monitoring BKV loads in the urine and serum or plasma of renal transplant recipients, therefore, may be valuable in identifying those at risk of developing BKVAN, allowing further investigation and early intervention if necessary [3, 9]. Such measurements are also valuable in monitoring response to therapy [3, 9].

Although suggested BKV load thresholds for quantitative molecular measurements vary, and laboratories are encouraged to establish their own cut off values for the purpose of clinical management [9], BK viral loads of greater than 10,000 copies/mL in blood [6, 11-14] and greater than 10 million copies/mL in urine are considered predictive for BKVAN [6, 11, 12].

Current guidelines recommend screening for BKV in the serum or plasma of kidney transplant patients monthly for the first 3-6 months after transplantation, and then every 3 months up to one year post-transplantation [15]. These guidelines also recommend that patients are screened for BKV if there is an unexplained rise in serum creatinine or following treatment for acute rejection [15].

Faster Quantification of BKV

Due to the specialist nature of BKV testing and the resources and expertise required to perform BKV measurements by urine cytology or nucleic acid testing, many centres are required to send samples to a reference laboratory for analysis. Some laboratories have adopted in-house polymerase chain reaction (PCR) BKV assays. These can be labour intensive, variable in terms of specificity for BKV, and may require further confirmatory testing on positive samples, which can cause significant delays and can potentially impact patient management.

A new molecular method is now available that can reduce the turnaround time for quantitative BKV results significantly and provide the high specificity required for making important clinical decisions about the management of renal transplant patients. The Diasorin Liaison® Iam benchtop instrument, with its small footprint and ease of operation, offers a cost effective and scalable solution for laboratories servicing renal transplant centres. Demonstrating no cross reactivity with other significant pathogens, including JCV, the Iam BKV assay provides reliable, quantitative results on the same day as sample receipt [16].

The Iam BKV assay uses loop-mediated isothermal amplification (Diasorin Q-LAMP) to measure BKV DNA in urine, plasma or serum. Unlike conventional LAMP technology, Q-LAMP is a rapid, real-time, fluorescent technique that allows quantitative analysis of individual or multiple targets in a single reaction [16].

Q-LAMP is based on the recognition of multiple primer binding regions on the target nucleic acid and amplification of the target sequence, which is facilitated by polymerase with strand displacement activity. Quantification is achieved through the use of fluorophore-labelled primers and an observed decrease in fluorescence during amplification of the target sequence, together with known calibrators. The Iam BKV assay is a duplex Q-LAMP assay, designed to recognise a consensus sequence common to all known BKV subtypes. Integral controls provide verification of the efficiency of the extraction process and demonstrate the absence of inhibitors [16].

The Iam BKV Q-LAMP assay fits easily into daily laboratory routines. Once samples are prepared and loaded onto the Liaison® Iam instrument, no operator intervention is required during an assay run, allowing staff to walk away until the routine is completed and the result is displayed. The Iam BKV assay is extremely sensitive, with a limit of detection (defined as that concentration of BK virus with a 95% probability of detection by probit analysis) of 450 cps/mL in plasma (95% Confidence Interval 350 – 770 cps/mL) and 540 cps/mL in urine (95% CI 440 – 780 cps/mL) [16]. The BKV primers used represent all known BKV subtypes (Ia, Ib-1, IB-2, Ic, II,III and IV) and show no significant homology with a range of pathogens, including SV-40 virus and Herpes viruses, or cross reactivity with the closely-related Polyomavirus, JCV [16].

Improved Management of Renal Transplant Patients

The Iam BKV assay for the detection and quantification of BKV has been in use at the 975-bed Westmead Hospital in Sydney since July, 2013. Westmead Hospital is a major teaching hospital for Sydney University and one of Australia's largest centres for post-graduate training to specialist level in all fields. The Department of Renal Medicine and Transplantation and the Centre for Transplant and Renal Research work closely with the Centre for Infectious Diseases and Microbiology (CIDM), which is part of Pathology West, a leading public pathology service in New South Wales. The focus of the Transplant and Renal Research Group is to improve the lives of people with end-organ failure through transplantation. It also aims to reduce the number of people requiring dialysis by preventing the progression of chronic renal disease.

Senior Hospital Scientist at the Westmead CIDM laboratory, Dr Neisha Jeoffreys, commented: "BKV is an important pathogen in renal transplant patients. It can cause serious complications and so early detection of viral reactivation and accurate monitoring of viral loads is a vital aspect of patient management."

The Westmead CIDM laboratory provides a BKV testing service to the hospital's renal transplant outpatient clinics as well as other specialist clinics associated with the centre. They also test samples from other pathology groups in their reference capacity. Dr Jeoffreys explained, "Renal transplant patients are tested routinely using the Iam BKV assay at 1, 2, 3, 6, 9 and 12 months post transplantation. Patients that test positive for BKV are tested more frequently, every 2-4 weeks.

"The role of the quantitative Iam BKV assay is to determine if the patient is likely to develop BKVAN, which may lead to premature graft loss. Patients with high BKV levels will have their immunosuppression regime modified in order to reduce BKV levels while preventing graft rejection. Ongoing monitoring of BK viral load then assists the renal physicians to ensure the right amount of immunosuppression is delivered to reduce the risk of BKVAN and maintain a healthy graft. Quantitative results allow the physicians to determine the appropriate point at which to modify the treatment."

Previously, the laboratory used a qualitative in-house conventional PCR method for the detection of BKV followed by monthly quantification of viral loads in BKV-positive patients using a commercially available real-time PCR assay.

"We feel that the Iam BKV assay enables us to provide a better service for our renal specialists," Dr Jeoffreys continued. "We like the scalability of the Liaison® Iam instrument. We have 3 instruments, which provide the flexibility to perform 1 or up to 21 samples at the same time, optimising reagent usage. This has allowed us to provide faster turnaround of results as we can now perform quantitative assays immediately and several times a week. The Liaison® Iam method has also helped to improve workflows as it is fast and easy to perform, with less hands-on time than our previous methods, which makes it more cost effective."

"It is hoped that the rapid quantitative results provided by the Iam BKV assay will allow our renal physicians to respond more quickly to high or escalating BK viral loads," Dr Jeoffreys concluded. "This will ultimately reduce the rate of graft loss due to BKVAN and allow for better patient management with reduced immunosuppression."

Dr Neisha Jeoffreys is Senior Hospital Scientist at the Centre for Infectious Diseases and Microbiology (CIDM) based at Westmead Hospital, part of the Pathology West Institute of Clinical Pathology and Medical Research (ICPMR).

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