Detection of Polyomavirus BK Reactivation After Renal Transplantation Using an Intensive Decoy Cell Surveillance Program Is Cost-Effective

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Background. Reactivation of polyomavirus BK (BKV) after renal transplantation can lead to allograft dysfunction or loss with early detection improving outcomes. Current guidelines recommend quantitative polymerase chain reaction for surveillance; however, urinary decoy cell detection is a potentially cost-effective alternative. We present the outcomes from an early intensive BKV surveillance program using decoy cell detection for initial screening starting 2 weeks after transplantation.

Methods. Records for all recipients of kidney (n = 211) or simultaneous kidney and pancreas (n = 102) transplants performed over 2 years in a single center were reviewed. Follow-up was for a minimum of 1 year. Urine cytology screening was performed fortnightly from 0 to 3 months after transplantation, monthly from 3 to 6 months then every 2 months from 6 to 12 months.

Results. Decoy cell positivity occurred in 56 of 313 patients (17.9%) with sustained decoy cell positivity (≥2 positive urine samples ≥2 weeks apart) present in 32 patients (10.2%). Twenty-four patients (7.6%) became viremic and three patients (1%) developed poloma virus nephropathy. The median time after transplantation until decoy cell positivity was 78 days, decreasing to 67 days for patients with sustained positivity and 57 days for patients who developed poloma virus nephropathy. No grafts were lost due to BKV during the study period. Decoy cell screening resulted in savings of approximately £135,000 over 2 years, when compared with routine surveillance by quantitative polymerase chain reaction.

Conclusions. Clinically significant BKV reactivation occurs early after transplantation and can be reliably detected by decoy cell screening. A surveillance strategy for detecting BKV reactivation based on urine cytology is cost-effective.

Keywords: Polyomavirus BK, Renal transplantation, Decoy cell.

Polyomavirus BK (BKV) is a ubiquitous small DNA virus that infects up to 90% of the adult population (1–3). After initial exposure in childhood, the virus becomes latent in urothelial tissues (4, 5) with intermittent asymptomatic shedding in the urine occurring even in healthy individuals throughout life (6). In immunosuppressed patients, loss of functional antiviral immunity can result in uncontrolled viral reactivation, which if unchecked can lead to a viral nephritis (polyomavirus nephropathy), causing allograft dysfunction or loss (7–12). Although the development of polyomavirus nephropathy (PVN) is considered a late complication of transplantation, often occurring after the first year (9, 13, 14), viral reactivation usually occurs much earlier than this (15–19). Prompt detection of reactivation can improve outcomes and is the basis for recommending routine surveillance after transplantation (9, 20, 21).

The detection of BKV reactivation in transplant recipients generally relies on one of two strategies: (1) recognition of viral cytopathic effect in infected urothelial cells (decoy cells) or (2) detection of viral nucleic acid in urine or blood (9). As the detection of decoy cells requires a trained cytologist to analyze samples and has a lower positive predictive value for the development of PVN than viremia (22), there has been a move toward nucleic acid assessment by polymerase chain reaction (PCR) as the screening modality of choice, endorsed by current guidelines (23, 24). However, these as-
sustained decoy cell positivity (defined as ≥2 positive samples >2 weeks apart) progressing to quantitative PCR (qPCR). This protocol has facilitated the introduction of an early intensive screening program for BKV reactivation starting 2 weeks after transplantation, whereas at the same time helping to minimize costs. The results of the first 2 years of this program are presented here.

**RESULTS**

**Characteristics of the Transplant Population**

Three hundred thirteen renal transplants were performed during the review period: 211 patients received kidneys alone (KT) and 102 patients simultaneous pancreas and kidney grafts (SPK). The average age of recipients was 46 years (range, 17–77 years) and donors 43 years (range, 1–80 years). One hundred fifty-nine patients received kidney transplants from deceased donors (40 non-heart beating) and 52 kidney transplants were performed from living donors. Recipients of organs from deceased donors were not more likely to develop decoy cell positivity (P=0.67). Consistent with published literature, patients who developed sustained decoy cell positivity were significantly older than patients who never became decoy cell positive (52.3 years vs. 45.6 years, P=0.005). Donor age, graft type (KT vs. SPK), number of HLA mismatches (or presence of the HLA-Cw7 allele), donor type (deceased vs. living donor), cytomegalovirus serostatus, type of induction agent (depleting vs. non-depleting antibody therapy), biopsy-proven rejection, and organ allocation (local vs. national) were not associated with the development of decoy cell positivity (P>0.05) (Table 1; see Table, SDC 1, http://links.lww.com/TP/A506). All recipients of SPKs received alemtuzumab induction and were managed with a steroid-free regimen consisting of tacrolimus and mycophenolate mofetil. Full details of the immunosuppressive regimens in use during the review period are shown in SDC 2 (see Table, http://links.lww.com/TP/A507).

**Intensive Decoy Cell Surveillance Program**

The early intensive decoy cell surveillance program was introduced after a previous study from our unit identified clinically significant viruria occurring relatively early after transplantation (29). This new protocol involved screening for decoy cells fortnightly from 2 weeks after transplantation, decreasing to monthly from months 3 to 6 then two monthly until 1 year (additional testing could be performed at the discretion of the treating clinician). Two thousand three hundred sixty-six urine samples were screened for the presence of decoy cells (mean of eight samples per patient). Adherence to the screening protocol was highest in the first 3 months after transplantation and declined thereafter. A total of 7.6% of patients did not have any recorded urine samples processed for decoy cells. SDC 3 (see Figure, http://links.lww.com/TP/A508) shows the number of samples received per patient.

To determine the incidence of viruria and viremia in our cohort, we reviewed the number of urine samples positive for decoy cells and the results of all qPCR analyses performed. Fifty-six patients (17.9%) had at least one urine sample positive for decoy cells with 32 patients (10.2%) demonstrating sustained decoy cell positivity. (For three patients with single positive samples, no subsequent samples were sent for decoy cell analysis; however, in each case, graft function remained stable.) In the sustained positive group, 24 patients (75%) had viremia proven by qPCR. Of the remaining eight patients, four had negative qPCR tests (<600 copies/mL) and four patients had no BKV qPCR results recorded. Three patients (1%) developed biopsy-proven PVN. Seventeen SPK recipients developed decoy cell positivity, of which eight had sustained decoy cell positivity and six became viremic. Thirty-nine KT recipients had positive urine samples, with 24 patients developing sustained decoy cell positivity. Eighteen became viremic. Concurrent pancreas transplantation was not associated with an increased likelihood of developing decoy cell positivity (P=0.75), sustained decoy cell positivity (P=0.23), or viremia (P=0.50).

**BKV Reactivation Occurs Relatively Early After Transplantation**

Consistent with previous reports (18, 29, 30), decoy cells were detected in some samples within a few weeks after transplantation, with the earliest positive samples received at day 14. The median time until decoy cell positivity after trans-
plantation was 78 days, decreasing to 67 days for patients with sustained decoy cell positivity and to 57 days for patients who developed PVN (Fig. 1). The median time to the development of PVN after transplantation was 149 days (mean=146 days; range, 121–170 days). PVN only developed in patients who were both decoy cell positive and viremic. Indications for biopsy included persistent viremia and worsening graft function. For patients with sustained decoy cell positivity, the median duration of decoy cell positivity was 133 days. The median time until detection of viremia was 105 days (range, 48–360 days) with viremia developing on average 45 days (range, 13–149 days) after viruria. The median duration of viremia was 94 days (range, 42–161 days) with viremia and decoy cell positivity usually resolving within 3 weeks of each other.

Changes in Immunosuppression in Patients With Sustained Decoy Cell Positivity

Sustained decoy cell positivity defines a group of patients who are unable to control BKV replication and are at increased risk of viremia and PVN (31). As plasma qPCR analyses are sent to an external provider and results are not available for at least several days, we next asked to what extent immunosuppression was adjusted by the treating clinicians once sustained decoy cell positivity was detected. Although the protocol specified that immunosuppression should only be reduced in the presence of viremia (in the absence of other findings consistent with excessive immunosuppression) previous studies have suggested sustained decoy cell positivity alone may be an indication for immunosuppression reduction (32). Changes in immunosuppression were calculated (for all patients) using the immunosuppressive index developed by Vasudev et al. (14), with one immunosuppressive unit equal to 5 mg prednisolone, 100 mg azathioprine, 2 mg tacrolimus, or 500 mg mycophenolate mofetil. The mean reduction in the immunosuppressive index during the period of decoy cell positivity was 1.98 immunosuppressive units (P<0.0001) (Fig. 2). Three patients with viremia developed steroid-sensitive biopsy-proven acute rejection (all Banff IB) after a reduction in their immunosuppression. No grafts were lost due to BKV during the study period. In patients who developed PVN, the average reduction in immunosuppression between the onset of sustained viruria and the diagnosis of PVN was 2.8 immunosuppressive units.

Allograft Outcomes in Patients With Sustained Decoy Cell Positivity

As BKV reactivation is associated with worse graft outcomes (23, 29, 33), we analyzed the effects of BKV reactivation on allograft function in our patients at 3 monthly intervals after transplantation. Patients with sustained decoy cell positivity had higher average creatinine values at all time points when compared with patients who were never decoy cell positive (open inverted triangles); however, these differences were not statistically significant (at 90 days, P=0.3441; at 180 days, P=0.3141; at 270 days, P=0.2239; at 365 days, P=0.3581). Mean±SEM is shown.

Cost of Decoy Cell Surveillance

Published guidelines advocate routine BKV surveillance by plasma qPCR assessment monthly for the first 3 to 6 months after transplantation. Allograft function was assessed at 90, 180, 270, and 365 days after transplantation. Results were included if they were within 20 days of these dates to make allowances for the timing of clinic visits. Where more than one result was available in this time period the closest was used. Patients with sustained decoy cell positivity (filled squares) had higher average creatinine values at all time points when compared with patients who were never decoy cell positive (open squares) and BKV viruria was 67 days with the earliest positive results recorded 2 weeks after transplantation.
months and then every 3 months until 1 year after transplantation (15). In our hospital, plasma BKV samples are sent to an external provider for qPCR analysis at a current cost of £86 per sample. Using the minimum screening schedule suggested (with monthly screening for only the first 3 months), would require six qPCR tests per patient in the first year after transplantation, a total cost of £516 pounds per patient. The expenditure over 2 years for the 313 patients in this study would therefore have been £161,508 (assuming full compliance with the screening schedule and no additional testing in patients who were subsequently found to be positive). In contrast, decoy cell detection costs our unit approximately £1 per sample, (including reagents and laboratory disposables), with an additional annual cost of £10,000 per annum to use a cytologist part-time to review the slides. If all patients had fully adhered to the intensive screening protocol, 12 decoy cell assessments would have been performed during the first posttransplant year, per patient, costing £23,756 (£3756 + £20,000) over 2 years. Including the cost of qPCR for the 32 patients with sustained decoy cell positivity (an additional £2752) results in a total of £26,508; a net saving of £135,000 to occur in more than 15% of transplant recipients (29, 30). Before the introduction of the intensive screening protocol in 2007, the incidence of decoy cell positivity in our transplant population was 29% (29). In this current cohort, the incidence had decreased to 18%. Possible explanations include a move toward lower trough tacrolimus targets (14) and the introduction of a protocol for more rapid weaning of corticosteroids (45). If a similar trend is seen in other units, the overall cost-effectiveness of decoy cell based surveillance strategies is likely to be further increased as the incidence of viremia decreases (34, 46).

In keeping with data from units that screen frequently for BKV, the median times until the detection of viruria and viremia in our study were 78 and 105 days, respectively (Fig. 1 (10, 16, 30, 47). In contrast, where less intensive surveillance strategies are used, the detection of viral reactivation tends to occur later (30, 47–50). The early detection of BKV reactivation in our cohort and consequent heightened surveillance of these patients is likely to explain the short median time until the diagnosis of PVN (149 days) when compared with studies using less intensive surveillance (13, 14, 52, 52).

A major consideration for the development and implementation of any screening program is its cost-effectiveness (53, 54). Although our study is limited by its retrospective nature, in our center the use of decoy cell screening after transplantation has proved to be an efficient and cost-effective way to identify BKV reactivation when compared with routine surveillance using plasma qPCR alone. Although some authors have argued that screening programs are not cost-effective when the incidence of PVN is low (35, 46), we would contest that the benefits of screening extend beyond simply predicting the development of PVN. BKV reactivation is considered to be a marker of excessive immunosuppression (2, 17, 22, 30) and as therapeutic drug levels are imperfect correlates of immune function (55, 56), the early detection of sustained viral replication can assist clinical decision making by identifying patients who may be at risk of immunosuppression-related complications.

In summary, we have described the outcomes of a working decoy cell surveillance program using intensive screening for the detection of BKV reactivation beginning 2 weeks after transplantation. This protocol enabled patients at risk of viremia to be identified early and by restricting quantitative assessment of viremia to this population resulted in significant cost savings.

### DISCUSSION

The management of BKV remains a significant problem after renal transplantation and despite the publication of guidelines, optimal surveillance strategies and the frequency with which screening should be performed continue to be debated (9, 15, 34, 35). As the efficacy of therapeutic agents that have been trialled for the treatment of PVN have demonstrated conflicting results (9, 36–40), the cornerstone of management remains early detection of BKV reactivation and cautious reduction of immunosuppression, with the aim of stimulating host-antiviral immune responses without increasing alloreactivity (9, 41). Surveillance programs to detect BKV reactivation after transplantation are therefore of great importance, as early detection of at risk patients provides an opportunity for more timely modulation of immunosuppression, potentially reducing the risk of rejection while still enabling control of viral replication (17, 18). Achieving this balance is difficult, as evidenced by the high rejection rates reported in some studies after immunosuppression reduction (13, 42).

The intensive early decoy cell-based surveillance program reported here was developed after our experience of clinically significant viruria occurring early after transplantation (29) and is broadly similar to that used by Schaub et al. (19). Although decoy cell screening requires a trained cytologist and is less sensitive than quantification of viral nucleic acid for the prediction of PVN (22), because viruria precedes viremia (10, 30, 16), the early detection of viruria increases the time available for clinicians to modulate immunosuppression. Consistent with previous data, decoy cell assessment provided on average, an additional 45 days warning before viremia was detected (16, 30), with progression to viremia occurring in 75% of patients with sustained decoy cell positivity. Decoy cell analysis also enables the detection of patients with reactivation of the related polyoma virus JC, which may be missed by PCR (43) but has been documented to occur in more than 15% of transplant recipients (32, 44).

An unexpected finding from our study was the relatively low incidence of BKV reactivation when compared with previous studies (29, 30). A review of decoy cell screening, BKV qPCR results and clinical outcomes was undertaken for recipients of all transplants performed between May 1, 2007, and April 30, 2009, in a single center after the introduction of an intensive early decoy cell surveillance program. The screening protocol required urine to be sent for cytological analysis fortnightly from 0 to 3 months after transplantation, monthly from 3 to 6 months then every 2 months from 6 to 12 months. Urine samples for decoy cell assessment were reviewed by a trained cytologist, with positive results verified by an independent histopathologist. Additional urine samples could be sent at the discretion of the treating clinician. Clinical outcomes, including rejection and graft function during the first year after transplantation were assessed through review of electronic and paper records. Patients were divided into three groups on the basis of decoy cell screening results: (1) no evidence of decoy cell positivity at any time during the first posttransplant year, (2) transient decoy cell positivity (not sustained), and (3) sustained decoy cell positivity: ≥2 urine samples
positive for decoy cells more than 2 weeks apart. According to the protocol patients with sustained decoy cell positivity had plasma sent for BKV qPCR analysis (a spare anticoagulated sample was routinely taken at each clinic visit and sent if there was a second positive urinalysis to ensure quantitation of viremia was not delayed until the next clinic appointment). Data for 29 patients were censored: 20 patients transferred to other units (18 decoy cell negative and 2 decoy cell positive), 8 patients died (all in the decoy cell negative group), and 1 graft failed (also in the decoy cell negative group), necessitating a return to hemodialysis. Patient data were censored from the time of transfer, death, or graft loss. Statistical analyses were performed in Prism Version 5 (GraphPad Software, Inc., La Jolla, CA).

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