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Prediction of Early BK Virus Infection in Kidney Transplant Recipients by the Number of Cells With Intranuclear Inclusion Bodies (Decoy Cells)

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Background. BK virus (BKV) is the cause of nephropathy. Because BKV nephropathy can progress to graft loss, early diagnosis of BKV infection is very important. In this study, we aimed to investigate the utility of quantifying cells with intranuclear inclusion bodies (decoy cells) in urinary sediment for the screening and monitoring of BKV infection in renal transplant recipients at our hospital. **Methods.** This was a retrospective single-center study. Urine sediment examination was performed at each outpatient visit, and the number of decoy cells was measured in the whole microscopic field. Patients (n = 41) were divided into the BK viremia group (blood positive for BKV DNA by polymerase chain reaction [PCR]) and non-BK viremia group (blood negative for BKV DNA by PCR), and the decoy cell count in urinary sediments was examined. **Results.** The maximum decoy cell count was significantly higher ($P = 0.04$) in the BK viremia group than in the non-BK viremia group. In the receiver operating characteristic curve for the maximum decoy cells, the cutoff value was 507 cells. The area under the receiver operating characteristic curve was 0.8774 (95% confidence interval, 0.7739-0.9810). The number of decoy cells at the time of appearance in the BK viremia group was not significantly different from that in the non-BK viremia group. However, the BK viremia group showed an increasing trend, whereas the non-BK viremia group showed a decreasing trend, in the number of decoy cells. There was a positive correlation between the number of decoy cells and the data from the urine BKV-DNA PCR quantification (correlation coefficient [r] = 0.74). **Conclusions.** Measurement of decoy cells in urinary sediments may predict early BKV infection, and if performed quickly, it may be useful for screening and continuous monitoring of BKV infection in renal transplant recipients.

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BK virus (BKV; polyomavirus BK) is an oncogenic virus of the papovavirus family that was first isolated in 1971 from the urine of a 39-year-old Sudanese male renal transplant recipient with ureteral stricture,[1] and it was recognized as a virus that could lead to ureteric stenosis and hemorrhagic cystitis in patients undergoing hematopoietic stem cell transplantation and in renal transplant recipients. BKV nephropathy (BKVN) was reported in renal transplant recipients for the first time in 1995.[2] BKVN was found to result in renal allograft damage in a background of more potent immunosuppressive regimens.[3] BKVN occurs in 10% of renal transplant recipients and can result in graft loss in up to 50% of those affected.[4,5]

Patients initially acquire BKV infection during childhood and develop transient viremia with cold-like symptoms, after which chronic BKV infection of the urinary system is established. The rate of antibody positivity in adults is around 90%,[6,7] but in adults with normal immunocompetence, BKV infection is latent and rarely causes disease. However, renal transplant recipients, who are subject to immunosuppressive therapy, are thought to have reactivated BKV infection due to this immunosuppression, resulting in chronic infection of the donor kidney.[8]

Patients develop BK viruria, in which the virus is found only in urine, during early reactivation of BKV. When infection ascends to the renal graft cortex, it leads to BK viremia and eventually BKVN. BK viruria is known as a precursor that leads to BK viremia within 6 to 12 weeks, and BK viremia is a known precursor that leads to BKVN within 2 to 6 weeks.[9] However, spontaneous remission has occurred in some patients who developed only BK viruria, or BK viremia, without progressing to BKVN for several months. Guidelines

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regarding BKV infection were issued by the Kidney Disease Improving Global Outcomes group and the American Society of Transplantation. These guidelines emphasize the detection of BK viremia before it progresses to BKVN and has a detrimental effect on graft function.[9-11] Because graft dysfunction is generally not found in patients with either BK viruria or BK viremia, diagnosis is difficult unless periodic screening is conducted. As a screening test, quantitative polymerase chain reaction (PCR) of blood is recommended in the guidelines of both Kidney Disease Improving Global Outcomes and the American Society of Transplantation. However, in many institutions, urinary cytology has excellent specificity and is used for screening; PCR testing of plasma is an additional screening technique used only if cells with intranuclear inclusion bodies (decoy cells) are continuously detected. Furthermore, because BKV-DNA PCR quantification is not covered by insurance in Japan, urinary cytology is used as a screening test in many institutions.

In urine cytology, decoy cells having antibasic intranuclear inclusion bodies with a ground-glass appearance are regarded as an important indicator of the asymptomatic reactivation of BKV. The sensitivity, specificity, positive predictive value, and negative predictive value to develop BKVN of the detection of these decoy cells are 100%, 71%, 29%, and 100%, respectively, which shows that their detection is very useful as a screening test.[12] It is important to detect BK viremia at an early, preliminary stage of BKVN. Urinary cytology and quantitative nucleic acid amplification (PCR quantification) in urine and blood are useful in the diagnosis of BKVN. In particular, BK viremia can be associated with BKVN.

Although qualitative tests in urine cytology can be used to ascertain the presence or absence of BKV infection, it is impossible to predict whether BK viremia will occur, and PCR quantification is needed to fully understand the status of the infection. In some patients, urinary cytology is positive for the presence of decoy cells, but BKV-PCR testing is negative. Although it has not been previously reported, it may be possible to determine the presence of BKV infection and thus the potential for BKVN by quantifying the presence of decoy cells in urine without the need for PCR testing.

We think this measurement can be a simple marker for the early detection of BK viremia. In this study, we investigated the utility of quantifying cells with intranuclear inclusion bodies (decoy cells) in urinary sediment for the screening and monitoring of BKV infection in renal transplant recipients.

MATERIALS AND METHODS

This was a retrospective single-center study. Of the 187 patients who underwent kidney transplantation in our hospital between April 2006 and December 2015, 41 patients (26 men and 15 women; mean age, 46.9 years) with decoy cells consistently present in their urinary sediment were enrolled in this study. Those patients in whom decoy cells were observed only once and then consecutively confirmed to be absent were excluded. BKV-DNA PCR of blood and urine was performed only when decoy cells were continuously detected. Urine was collected midstream during outpatient visits, and the 314 urine samples obtained from the 41 patients were prepared for examination as follows. Samples were centrifuged in 10-mL centrifuge tubes (Sanplatec Corporation, Osaka City, Japan) at 500*g* for 5 minutes, after which 15 μL of the sample was pipetted onto a glass slide and cover-slipped. The slide was then placed on a light microscope, and the entirety of the cover-slipped area was observed at 200× magnification to perform a count of decoy cells. The slides were then observed at 400× magnification to double-check the accuracy of the previous count. Cells with the following findings were considered to be decoy cells: (1) nuclear hypertrophy, (2) high nucleus-to-cytoplasm ratio, (3) thick nuclear membrane, (4) homogeneous nucleus (ground-glass or transparent appearance), (5) watery surface profile, (6) appearance of intranuclear inclusions (irregular shape and size or homogeneous globular configuration), (7) narrow cytoplasm or bare nucleus, and (8) appearance of nuclear decay. Both stained (Sternheimer stain) and unstained slides were observed. Decoy cell counts were performed twice on the whole field of view on light microscopes, both right after staining and at 15 to 20 minutes thereafter. BKV DNA-PCR was performed to examine the association between decoy cells and the presence of the virus in either blood or urine.[10]

In total, 75 urine and 75 plasma samples were obtained from the 41 patients. BKV-DNA PCR positivity is indicated by a BKV load greater than 4 log copies/mL in blood and by a BKV load greater than 7 log copies/mL in urine.[13] Before DNA extraction, the blood samples were diluted twofold in 1× PBS buffer, boiled for 10 minutes, and then centrifuged at 13 000*g* for 15 minutes at room temperature. DNA was extracted from the urine and blood samples as follows: 1 mL of urine and/or supernatant of boiled sera was added to 1 mL of lysis buffer (400 mM Tris-HCl, pH 7.5, 500 mM NaCl, 50 mM ethylenediaminetetraacetic acid, 1% sodium dodecyl sulphate), incubated at room temperature for 90 minutes, then centrifuged at 13 000*g* for 5 minutes. The supernatant was mixed with an equal amount of isopropanol and incubated at −80 C for 30 minutes. After centrifugation at 13 000*g* for 5 minutes, the pellet was washed with 70% ethanol, centrifuged again at 13 000*g* for 5 minutes, and then dried and resuspended in 20-μL ultrapure H_2O.

Samples were selected in a semi-quantitative PCR assay based on amplification in separate tubes of samples and serial dilutions of an external standard (4 tenfold dilutions of pBK385 containing from 10^2 to 10^5 copies). To check the reliability of the external standard and to identify negative results due to potential Taq polymerase inhibitors in the samples, the 4 dilutions of the external standard and each sample were coamplified in the presence of 100 copies of pBK-C in each assay. Viral copy number was determined by comparing the intensity of the band of the sample with that of bands from known amounts of pBK385. Samples estimated to contain 10^3/mL or greater BKV-DNA copies were then quantified in a quantitative-competitive PCR assay in which they were coamplified with 6 dilutions of competitor pBK-C containing 1000, 5000, 10 000, 50 000, 100 000, and 500 000 copies. The copy number of the competitor giving a 356-bp band with an intensity equal to that of the 278-bp band was determined and regarded as the copy number of the BKV-DNA in the reaction.

The subjects were subsequently assigned to 1 of 2 groups: blood BKV-DNA PCR-positive (BK viremia group) or blood BKV-DNA PCR-negative (non-BK viremia group). The following parameters were subsequently determined for each patient: the maximum decoy cell count, the trend of decoy cell counts after the appearance of the decoy cells, and the first time to decoy cell detection after transplantation. Statistical analysis was performed using BellCurve for Excel (Social

TABLE 1.
Clinical characteristics of the patient groups

Characteristics	BK viremia (n = 10)	Non-BK viremia (n = 31)	*P*
Age at transplantation, y	54.3 ± 12.3 [a]	44.5 ± 12.9	0.04
Male sex	7 (70%)	19 (61.3%)	0.68
Body mass index, kg/m^2	21.5 ± 2.9	22.1 ± 3.7	0.66
Primary kidney disease			0.59
Chronic glomerulonephritis	7 (70%)	25 (80.6%)	
Diabetic nephropathy	0	3 (9.7%)	
Urological diseases	2 (20%)	2 (6.5%)	
Unknown	1 (10%)	1 (3.2%)	
Renal replacement therapy			0.45
Hemodialysis	8 (80%)	20 (64.5%)	
Peritoneal dialysis	0	3 (9.7%)	
Preemptive [b]	2 (20%)	8 (25.8%)	
Donor characteristics			
Age, y	60.4 ± 9.1	54.4 ± 12.5	0.16
Male sex	3 (30%)	11 (30%)	0.83
Type			0.63
Live related	8 (80%)	28 (90.3%)	
Live unrelated	2 (20%)	3 (9.7%)	
ABO-incompatible	4 (40%)	9 (29%)	0.63
HLA-AB, DR mismatches	2.5 ± 1.2	3.27 ± 1.72	0.36
0	1 (10%)	0	
1-3	7 (70%)	21 (67.7%)	
4-6	2 (20%)	10 (32.3%)	
Immunosuppression			0.77
Tacrolimus	9 (90%)	26 (83.9%)	
Cyclosporine	1 (10%)	5 (16.1%)	
Antimetabolite			0.45
Mycophenolate mofetil	10 (100%)	26 (83.9%)	
Azathioprine	0	5 (16.1%)	
Serum creatinine, mg/dL	1.57 ± 0.4	1.49 ± 1.19	0.14
Ureteral stent			0.3
None	4 (40%)	17 (54.8%)	
Yes	6 (60%)	14 (45.2%)	
Ureteral stent duration, d	15.7 ± 7.7	10.2 ± 3.8	0.39
Rejection	2 (20%)	7 (22.6%)	0.68

[a] Values are mean ± SD or number (%).
[b] Preemptive kidney transplantation.

Survey Research Information Co., Ltd., Tokyo, Japan). The Mann-Whitney *U* test was performed to determine statistical significances between the 2 groups, and significance was set at *P* less than 0.05. Data collection and statistical analysis were conducted after the approval of this study by the local institutional ethical review board (approval number 28-338).

RESULTS

The BK viremia group comprised 10 patients, and the non-BK viremia group comprised 31 patients. There was a significant difference in the age at transplantation between the 2 groups (*P* = 0.04); however, no significant differences were found in the other characteristics between the 2 groups (Table 1).

The mean (±SD) maximum decoy cell count was significantly higher in the BK viremia group than in the non-BK viremia group (1645.1 ± 2326.6 cells; median, 532 cells; range, 98-7500 cells vs 651.8 ± 825.5 cells; median, 380 cells; range, 2-2770 cells; *P* = 0.04; Figure 1).

In the receiver operating characteristic (ROC) curve for the maximum decoy cell count, the cutoff value was 507 cells. The area under the ROC curve was 0.8774 (95% confidence interval [CI], 0.7739-0.9810; Figure 2).

No significant difference was found between the BK viremia group and the non-BK viremia group in the number of decoy cells at the time of appearance (204.5 ± 220.7 cells; median, 104 cells; range, 26-744 cells vs 376.3 ± 468.3 cells; median, 140 cells; range, 5-2086 cells; *P* = 0.52).

All patients in the BK viremia group showed an increasing trend in the number of decoy cells, whereas all patients in the non-BK viremia group showed a decreasing trend (Figures 3 and 4). There was also no significant difference between the 2 groups in the first time to decoy cell detection after transplantation (Figure 5). There was positive correlation

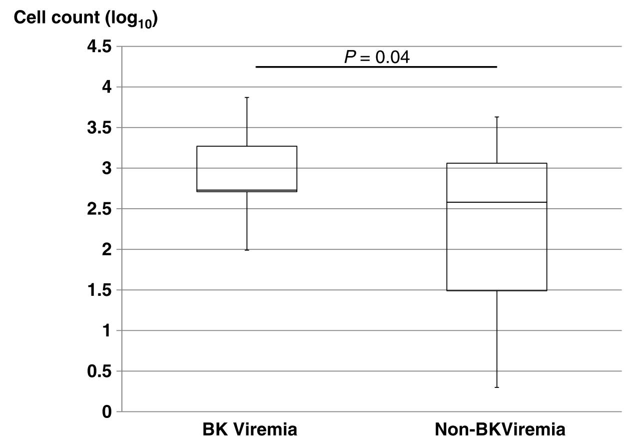

FIGURE 1. Maximum count of the decoy cells. There was a significant difference in the mean (±SD) maximum count of decoy cells between the BK Viremia group and the Non-BK Viremia group (1645.1 ± 2326.6 cells; IQR, 507-1880 cells; median, 532 cells; range, 98-7500 cells vs 651.8 ± 825.5 cells; IQR, 31-1056 cells; median, 380 cells; range, 2-2770 cells; *P* = 0.04). IQR, interquartile range.

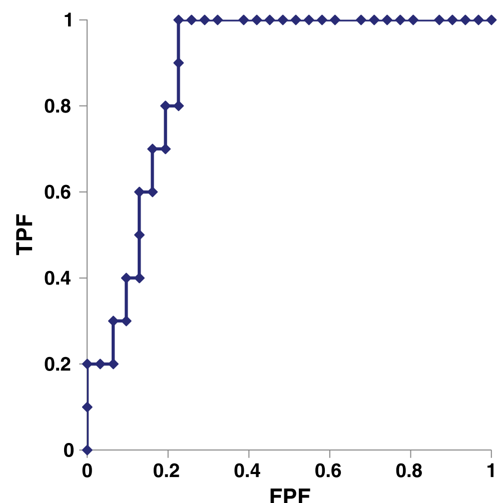

FIGURE 2. ROC curve for the maximum count of decoy cells. The cutoff value for the maximum count of decoy cells was 507 cells. The area under the ROC curve for the maximum count of decoy cells was 0.8774 (95% CI, 0.7739-0.9810). TPF, true-positive fraction; FPF, false-positive fraction.

between the number of decoy cells and the data from urine BKV-DNA PCR quantification (correlation coefficient [r] = 0.74) (Figure 6).

DISCUSSION

Risk factors for BKV infection include increased age of both the recipient and donor, male recipient, receiving a kidney from a female donor, placement of ureteral stents, human leukocyte antigen mismatch, and a history of rejection.[14-16] There was a significant difference in the mean age of the recipients in this study, and being an elderly recipient was a risk factor for BKV infection. Although no significant difference was found between the 2 groups in terms of male recipient as a risk factor for BKV infection, more than half of the subjects in each group were male recipients; thus, male recipient as a potential risk factor for BKV infection should be kept in mind. In addition, more than half of the donors were female in both groups. The mean age of the donors was also not significantly different between the 2 groups, but on average, the age of the patients in the BK viremia group was 6 years older than that in the non-BK viremia group. Although the difference was not significant, there were many elderly female donors in the BK viremia group. The immune system changes with age, which is related to a decrease in T-cell function. In addition to aging, immunosuppressive therapy can cause infection to occur more easily, so we believe that BKV infection can occur more commonly in the elderly.[17]

Ureteral stents are useful for avoiding the risk of urinary complications, but the uroepithelium is damaged by the mechanical stimulation of these stents, which can increase the risk of BK viremia.[16] In this study, patients were stented only during transplant surgery. There were no significant differences between the 6 patients in the BK viremia group and the 17 patients in the non-BK viremia group who received stents (P = 0.3), nor was there a significant difference in the duration of ureteral stent placement (15.7 ± 7.7 days in the BK viremia group and 10.2 ± 3.8 days in the non-BK viremia group; P = 0.39). Although stent placement resulted in no significant differences in the present study patients, long-term ureteral stent placement may cause BK viremia.

The renal replacement therapy administered before transplantation included hemodialysis in 8 patients and preemptive kidney transplantation in 2 patients in the BK viremia group and hemodialysis in 20, peritoneal dialysis in 3, and preemptive kidney transplantation in 8 patients in the non-BK viremia group. Although it is reported that hemodialysis can cause reactivation of the BKV and the risk of BK viremia,[18] there were no significant differences in renal replacement therapy before transplantation in the present study (P = 0.45).

The cutoff value by the ROC curve analysis for the maximum decoy cell count was 507 cells, and if 507 cells or more appear, BK viremia can be suspected. The area under the ROC curve was 0.8774 (95% CI, 0.7739-0.9810), indicating that the discrimination performance of the inspection was moderately accurate. Thus, BK viremia can be predicted when the number of decoy cells is approximately 500 cells or more. Because the decoy cells count after the appearance of the decoy cells tended to increase in all of the patients with BK viremia, it can be predicted that BK viremia will develop in patients

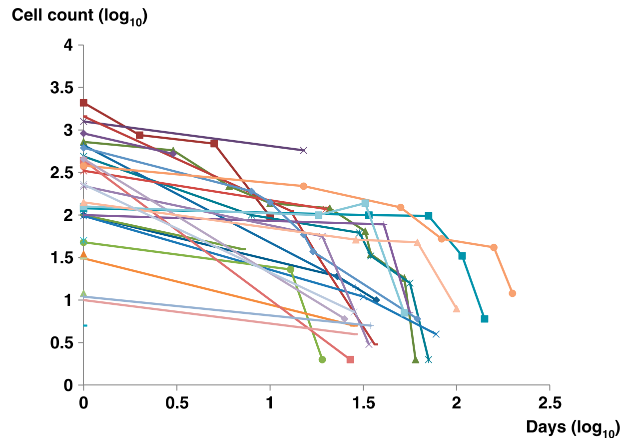

FIGURE 4. Change in the number of decoy cells in non-BK viremia. All cases in the non-BK viremia group showed a decreasing trend in the number of decoy cells from the time of their appearance.

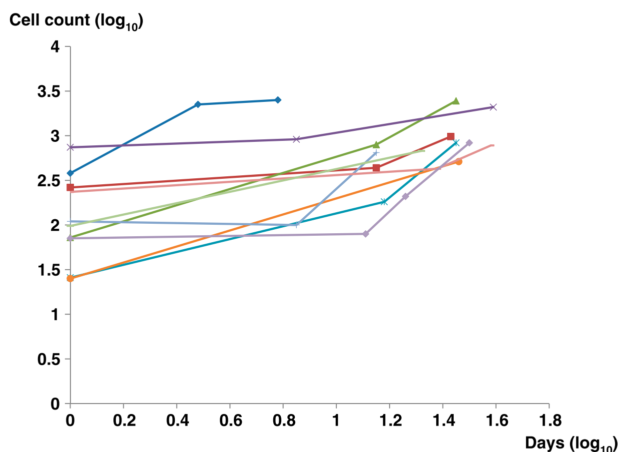

FIGURE 3. Change in the number of decoy cells in BK viremia. The BK viremia group showed an increasing trend in the number of decoy cells over time. Although some cases in this group showed an increasing trend in the number of decoy cells from the time of their appearance and others showed an increasing tendency after a transition without significant change, all cases showed an increase in the number of decoy cells present.

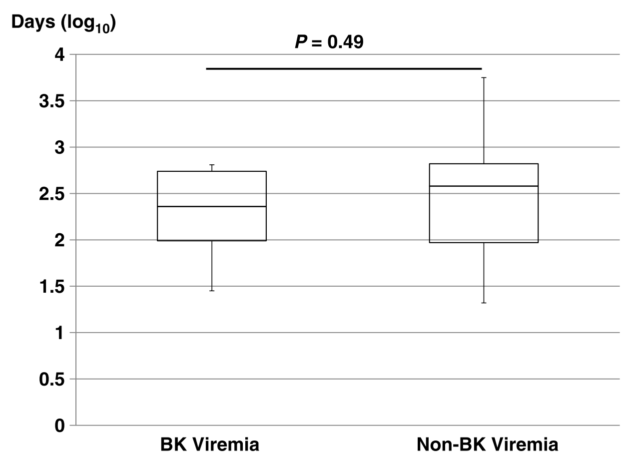

FIGURE 5. Time to detection of decoy cells after transplantation. There was no significant difference between the groups in time to detection of decoy cells after transplantation (300.2 ± 233.8 days; IQR, 98-553 days; median, 231 days; range, 28-645 days vs 690.4 ± 1161.3 days; IQR, 93-661 days; median, 378 days; range, 21-5623 days; P = 0.49).

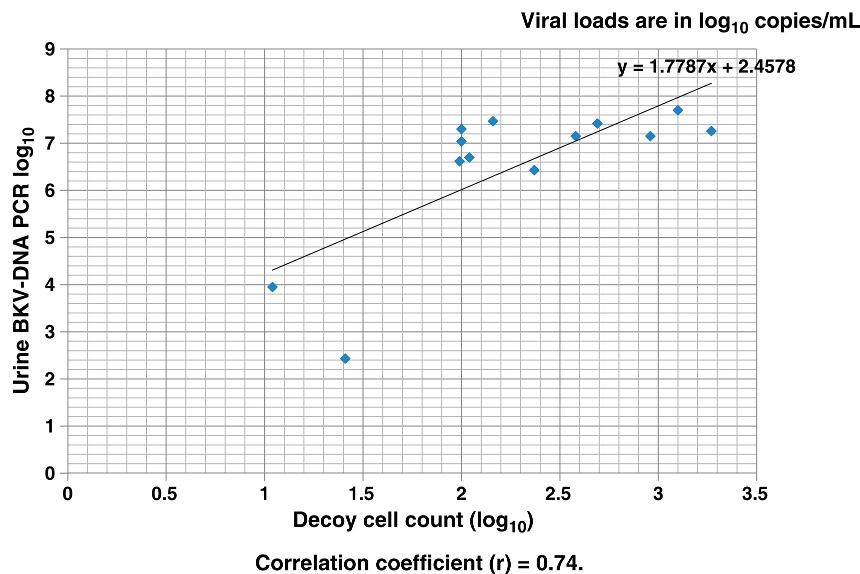

FIGURE 6. Correlation between the number of decoy cells and the data of urine BKV-DNA PCR quantification. There was a correlation between the count of the cells with intranuclear inclusion bodies and the data from urine BKV-DNA PCR quantification (correlation coefficient [*r*] = 0.74).

showing an increasing trend. In contrast, patients in whom the decoy cell count decreases can be predicted not to progress to BK viremia. However, it may not be possible to predict BK viremia based on the number of decoy cells at the first time of appearance because the number of decoy cells at this time was not significantly different between the 2 groups.

In this study, there was positive correlation between the decoy cell count and the data from urine BKV-DNA PCR quantification. Measurement of decoy cells may predict the early detection of BK viruria, which can lead to a better understanding of the conditions of patients who may progress to BK viremia and to the prevention of BKVN that progresses to graft loss. The trend in many patients was for the blood and urine BKV-DNA PCR results to become positive after the decoy cells appeared, for the BKV-DNA PCR results to then decrease as the number of decoy cells decreased, and finally for the decoy cells to disappear before the blood and urine BKV-DNA PCR results became negative.

Measurement of decoy cells in urinary sediment can be performed easily, economically, and quickly, and it is useful for the screening and continuous monitoring of BKV infection in renal transplant recipients. Based on the transition of the decoy cell count measured in urinary sediment over time at every outpatient visit, it may be possible to predict patients who will be progression to BK viremia.

There are some limitations of our study. First, this study lacked both a comparison of decoy cell counts with the BKV-DNA PCR results for determining BKV and the pathologic findings of the patients' grafts. Second, blood/urine BKV-DNA PCR measurements were performed in all cases at the time of decoy cell appearance, but after their appearance, the measurement period varied on a case-by-case basis. Third, the sample size is small, and all patients were Japanese.

CONCLUSIONS

BKVN is a factor affecting the prognosis of renal allograft patients that needs to be diagnosed at an early stage. BK viremia may be predicted from the measurement of decoy cells. To our knowledge, there are no reports regarding the quantification of cells with intranuclear inclusion bodies, that is, decoy cells. Measurement of decoy cells in urine sediment, which can be performed economically, easily, and quickly, may be a useful test for the screening and continuous monitoring of BKV infection.

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