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ORIGINAL ARTICLE

Clinical usefulness of BK virus plasma quantitative PCR to prevent BK virus associated nephropathy

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Summary

The present study investigated the clinical usefulness of plasma real-time polymerase chain reaction (PCR) (plasma-PCR) in the prevention of BK virus-associated nephropathy (BKVAN). First, we investigated the diagnostic value of plasma BK-PCR, urine BK-PCR, and urine cytology for the prediction of BKVAN retrospectively. Then we designed a prospective study of regular plasma-PCR monitoring and pre-emptive immunosuppression (IS) reduction based on the result. In the retrospective cohort, the prevalence of BKVAN was 3.7% (14/379) and the positive rate of decoy cells, urine-PCR ($>1 \times 10^{10}$ copies/ml), and plasma-PCR ($>1 \times 10^{4}$ copies/ml) was 18.6%, 11.1%, and 5.5%, respectively. Plasma-PCR was superior to urine-PCR or urine cytology in specificity and positive predictive value for detection of BKVAN. In prospective study, regular monitoring of plasma-PCR detected significant BKV viremia in 8.3% (12/145) and BKVAN in 1 patient (0.6%). After IS reduction, BKV viremia was eliminated in 91.6% (11/12) within 103 days (25–254). In patients with viremia, the frequency of acute rejection did not increase and allograft function did not differ significantly compared with those in patients without viremia during the first year post-transplant ($P > 0.05$, in both). Plasma-PCR is useful to predict an increased risk for BKVAN, and regular monitoring is effective to prevent the development of BKVAN.

Introduction

BK virus (BKV) occurs worldwide, with a seroprevalence rate of 60–90% in adults [1]. Following primary infection in childhood, BK remains latent in the renal tubules and uroepithelial cells, the epidemiologically most important sites [2]. In renal transplant recipients experiencing immunosuppression (IS), BKV can be activated, usually during the first year following transplantation [3,4].

Before noninvasive diagnostic tests for BKV replication were introduced, BK virus-associated nephropathy (BKVAN) was mostly diagnosed late in an advanced stage when irreversible functional damage had already occurred, leading to allograft loss in as many as 90% cases [5–9]. In these patients, histological examination of the allograft biopsy specimens revealed extensive replication of the BKV, cell necrosis in the tubules and collecting ducts, and varying degrees of interstitial inflammation [10,11]. In addition, there is no proven therapy other than IS reduction for treatment of BKV infection [12]. Therefore, on-time detection of BKV replication and prompt reduction of IS is recommended to prevent the development of BKVAN [13].

Because BKV replication exhibits a viruria–viremia–nephritis sequence, urine-based assays can detect BKV

replication faster than the plasma-based assays [14]. However, urine-based assays demonstrate a low positive predictive value (PPV), because most cases of BK viruria do not progress to BK viremia or nephritis [15]. In this respect, BK viremia, rather than viruria, is regarded as the prerequisite for BKVAN and it may provide opportunity for IS reduction in previous reports [16]. In the early 2000s, a real-time polymerase chain reaction (PCR) using plasma samples (plasma-PCR) was introduced for detecting BKV replication. Despite its accuracy and ability to quantitate viral loads, its clinical usefulness as a first-line screening method has not been fully investigated [17–20]. Furthermore, no clinically significant plasma viral load has been established [4,21,22].

Therefore, the first purpose of this study was to evaluate the prevalence of BK viremia, viruria, and nephropathy in renal transplant recipients, and to determine the diagnostic value of plasma PCR in the diagnosis of BKVAN in those patients. Second, this study aimed to investigate the safety and effectiveness of prospective IS reduction based on plasma PCR results for the prevention of BKVAN in another cohort.

Subjects and methods

Retrospective investigation of BKV replication and BKV nephropathy

Between January 2001 and September 2008, 376 kidney transplantations (KTs) were performed in Seoul St Mary's Hospital. We investigated the prevalence of BKVAN in this patient population. Between October 2006 and September 2008, 199 allograft biopsies were performed in 184 renal transplant recipients. The indication for allograft biopsy was the elevation of serum creatinine (Scr) 20% above baseline. Urine cytology, plasma, and urine BKV PCR examinations were done concurrent with allograft biopsy. Early morning urine and plasma samples were collected for examining BKV replication 1 day after allograft biopsy. We included those 199 cases for the evaluation of the diagnostic value of plasma and urine PCR, and urine cytology for the detection of BKVAN.

The diagnosis of BKVAN was confirmed when the following criteria were met: (1) typical histological features suggestive of BKVAN such as presence of intra-nuclear viral inclusions, (2) positive SV40T by immunoperoxidase staining, and (3) detection of BKV replication in at least one test among urine cytology, urine PCR, or plasma PCR. The histological stage of BKVAN was determined according to the classification described in a previous study [23].

BKV-DNA quantitation was performed by RT TaqMan PCR (Applied Biosystems, Foster City, CA, USA) to detect the target viral capsid protein (*VP-1*) gene encoding BKV by using an ABI PRISM 7000 real-time PCR system (Applied Biosystems). Urine cytology samples were stained by the Papanicolaou method and observed for the presence of urine decoy cells (characterized by a ground-glass appearance with an enlarged nucleus, which is occupied by a basophilic inclusion surrounded by chromatin). Decoy cell analysis was performed by a well-trained pathologist blinded to the results of allograft biopsy and plasma/urine PCR.

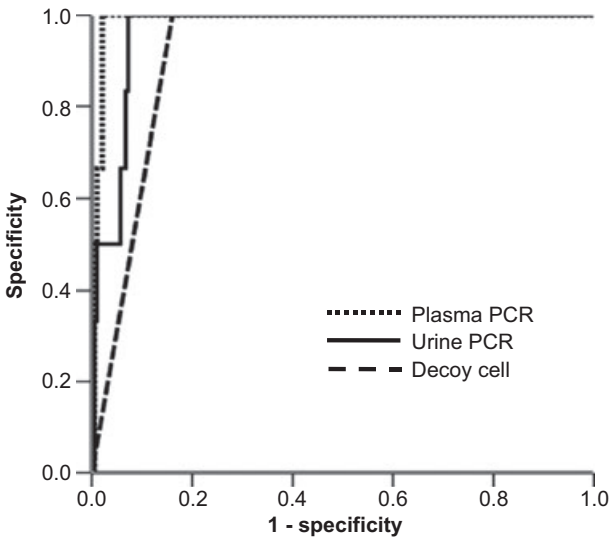

Figure 1 Receiver-operating characteristic plot analysis in plasma-PCR, urine-PCR, and urine decoy cell using the development of BKVAN. The area under the curve for plasma-PCR, urine-PCR, and urine decoy cell was 0.989, 0.964, and 0.920, respectively. The cut-off chosen was 1×10^4 copies/ml for plasma-PCR (sensitivity, 100%; specificity, 97.4%) and 1×10^{10} copies/ml for urine-PCR (sensitivity, 100%; specificity 91.8%). Plasma-PCR, plasma BKV real time PCR; urine-PCR, urine BKV real time PCR; BKVAN, BK virus associated nephropathy.

In receiver operating characteristic curve (ROC) analysis, 1×10^4 copies/ml by plasma PCR and 1×10^{10} copies/ml by urine PCR showed the highest sensitivity and specificity for the diagnosis of BKVAN. Therefore, values exceeding 1×10^4 and 1×10^{10} copies/ml in plasma and urine PCR were regarded as significant viremia or viruria, respectively (Fig. 1). We investigated the diagnostic value (i.e. sensitivity, specificity, PPV, and negative predictive value [NPV]) of each method including urine cytology, urine PCR, and plasma PCR for the diagnosis of BKVAN.

Prospective monitoring of BKV viremia using plasma BKV real-time PCR

We tested prospective BKV monitoring and an IS reduction protocol based on the results of plasma PCR in the prospective cohort. One hundred and forty-five renal transplant recipients who underwent KT between October

2008 and September 2010 were included in this prospective monitoring. The protocol was as follows: Plasma PCR was performed 1, 3, 6, 9, and 12 months after transplantation, and we evaluated the incidence of BKV viremia during the first year post-transplantation. Transient viremia meant that significant viremia ($>1 \times 10^4$ copies/ml) was detected only once. Sustained viremia denoted that significant viremia was detected two or more times.

Management of significant BKV viremia during prospective monitoring

In the event of viremia exceeding the cut-off value, which was determined in the retrospective cohort (1×10^4 copies/ml), mycophenolate mofetil (MMF) was discontinued. We rechecked plasma PCR 1 month later; if significant viremia was still detected (sustained viremia), we performed allograft biopsy, and the dose of calcineurin inhibitor (CNI) was reduced by 20%. We restarted MMF after the elimination of BKV viremia (Fig. 2).

Together with plasma PCR, we measured the Scr level and calculated the estimated glomerular filtration rate (eGFR) using the four-variable modification of the diet in renal disease (MDRD) formula: $eGFR = 186.3 \times Scr^{-1.154} \times age^{-0.263} \times 0.742$ if female [12]. The trough levels of cyclosporine (CsA) and tacrolimus (Tac) during the study period were also measured. The clinical progress according to the graft function and drug levels, and the development of acute rejection in patients with BKV viremia was compared with those observed in patients without BKV viremia. This study was approved by the Institutional Review Board of Seoul St. Mary's Hospital (KC11RISI0537).

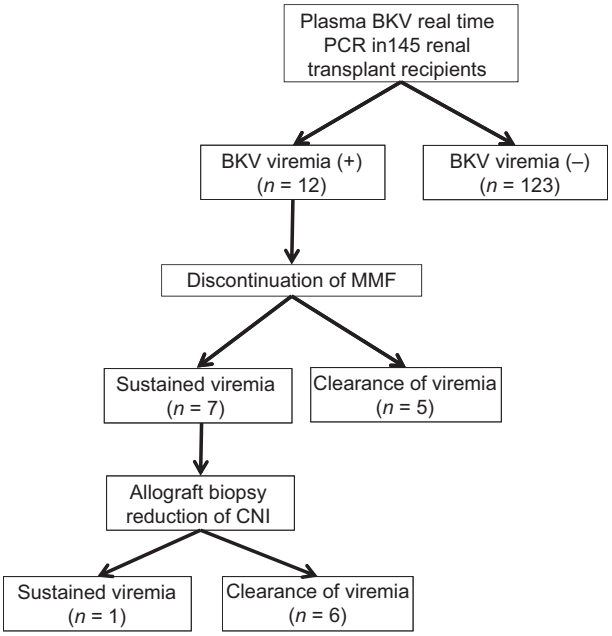

Figure 2 Patient flow algorithm in prospective BK virus real time PCR monitoring. Plasma BKV real time PCR was done at 1, 3, 6, 9, and 12 months after transplantation. In case of viremia higher than 1×10^4 copies/ml, immuno suppressive therapy was decreased. At first, MMF was discontinued, and if viremia sustained at 1 month later, allograft biopsy was done and CNI dose was reduced by 20%. In Using this protocol, BKV viremia was successfully cleared out in 11 of 12 cases. MMF, mycophenolate mofetil; CNI, calcineurin inhibitor.

Statistical analysis

Statistical analyses were performed using SPSS software (version 15.0; SPSS Inc., Chicago, IL, USA). Data are presented as mean ± SD or counts and percentages depending on the data type. For continuous variables, means were compared using Student's *t*-test. For categorized variables, Pearson's χ^2 test and Fisher's exact test were used. ROC analysis was used to investigate the prediction of development of BKVAN. All tests were two-tailed, and the results were considered significant at $P < 0.05$.

Results

Prevalence of BKV infection and BKVAN in the retrospective cohort

In the 376 patients who underwent KT between January 2001 and September 2008, 14 cases of BKVAN developed; hence, the prevalence rate was 3.7% (Table 1). In the 199

Table 1. The baseline characteristics of RTR in retrospective cohort and prospective cohort.

	Retrospective cohort (*n* = 376)	Prospective cohort (*n* = 145)	*P*
Age at KT (year)	43.5 ± 16.5	44.9 ± 10.7	0.33
Gender; male, *n* (%)	205 (54.5)	78 (53.8)	0.50
Retransplantation, *n* (%)	23 (6.1)	15 (10.3)	0.11
HLA mismatch number	3.4 ± 1.3	3.4 ± 1.8	0.17
ABO incompatibility, *n* (%)	0 (0.0)	9 (6.2)	<0.01
Deceased donor, *n* (%)	48 (12.8)	47 (32.4)	<0.01
Delayed graft function, *n* (%)	35 (9.3)	10 (6.9)	0.24
Main Immune suppressant, *n* (%)			
Cyclosporine	230 (61.2)	13 (9.0)	<0.01
Tacrolimus	145 (38.6)	132 (91.0)	
*Desensitization before KT, *n* (%)	3 (0.8)	12 (8.3)	<0.01
BKVAN (*n*, %)	14 (3.7)	1 (0.6)	0.05

RTR, renal transplant recipient; KT, kidney transplantation; BKVAN, BK virus associated nephropathy.

*Desensitization protocol composed of plasmapheresis, intravenous immune globulin and rituximab.

patients who underwent allograft biopsy concurrently with screening for BKV replication, the prevalence of positive decoy cells was 18.6% (37/199), significant viruria ($>1 \times 10^{10}$ copies/ml) was 11.1% (22/199), and significant viremia ($>1 \times 10^{4}$ copies/ml) was 5.5% (11/199). In those biopsy cases, six cases were diagnosed as BKVAN (3.0%).

Diagnostic value of plasma PCR for the diagnosis of BKVAN

In ROC analysis, the area-the-under curve values of plasma PCR, urine PCR, and urine decoy cells were 0.989, 0.964, and 0.920, respectively (Fig. 1). In the prediction of BKVAN, all three methods showed excellent sensitivity (100%) and NPV (100%). However, plasma PCR was superior to urine PCR and urine decoy cells with respect to specificity and PPV. The specificity and PPV of plasma PCR were 97.4% (188/193) and 54.5% (6/11), respectively. In contrast, the specificity and PPV of urine PCR were 91.7% (177/193) and 27.3% (6/22), respectively; whereas, the specificity and PPV of decoy cells were 83.9% (162/193) and 16.2% (6/37), respectively (Table 2).

Clinical progress of BKVAN in the retrospective cohort

In the 14 cases diagnosed as BKVAN in the retrospective cohort, the duration from transplantation to the diagnosis of BKVAN was 26.0 months (11.8–37.3 months). During the follow-up period, four cases of allograft failure developed at 6.2 months (1.0–16.2 months) after the diagnosis of BKVAN. In those patients, the Scr level at biopsy was 7.0 ± 0.7 mg/dl, and the biopsy specimen indicated BKVAN stage C in all four cases. In the remaining 10 patients, the Scr level at the time of biopsy was 2.6 ± 1.2 mg/dl; the BKVAN was stage A, B1, and B2 in two, six, and two patients, respectively. Renal function did not recover to the level before the development of BKVAN (Scr at last visit, 2.4 ± 0.6 mg/dl; 43.8 months, 25.7–71.1).

Table 2. Predictive value for the diagnosis of BKVAN of three diagnostic methods for BKV replication.

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Urine decoy cell	100	81.7	16.2	100
Urine-PCR	100	91.8	27.3	100
Plasma-PCR	100	97.4	54.5	100

BKVAN, BK virus associated nephropathy; PPV, positive predictive value; NPV, negative predictive value; Urine-PCR, urine BKV real time PCR; plasma-PCR, plasma BKV real time PCR; PCR, polymerase chain reaction.

Prospective monitoring using BKV plasma real-time PCR and BKVAN development

Seven hundred and seventy-five plasma samples were collected from 145 patients for plasma PCR during the first year post-transplantation. The median follow-up period was 19.5 months (12.1–32.2 months). Significant viremia ($>1 \times 10^{4}$ copies/ml) developed in 12 patients (8.3%): seven cases of sustained viremia and five cases of transient viremia (Fig. 2). Significant viremia developed at 163 days (29–287 days) from KT, and the peak DNA copy number was 3.3×10^{4} copies/ml (1×10^{4}–1.0×10^{6}). In the five patients with transient viremia, only MMF was discontinued for 1 month; in the seven patients with sustained viremia, the CNI dose was reduced by 20% in addition to the discontinuation of MMF. After IS reduction, viral load gradually reduced (Fig. 3); within 103 days (25–254 days), it was successfully cleared out in 11 patients (90.9%) (Table 3). No episodes of acute rejection were noted after the initiation of IS reduction in the viremia group.

Allograft biopsy findings at the time of BKV viremia

Allograft biopsy was performed in seven patients who showed sustained viremia. SV40T IHC was negative, and no morphological evidence of BKVAN was detected in any of the biopsy specimens. In all seven patients, acute CNI toxicity was suspected; in two of these patients, borderline tubulitis and interstitial infiltration were observed along with CNI toxicity. One patient was diagnosed with acute T cell-mediated rejection IA; steroid pulse therapy was applied and immunosuppressant dosages were not

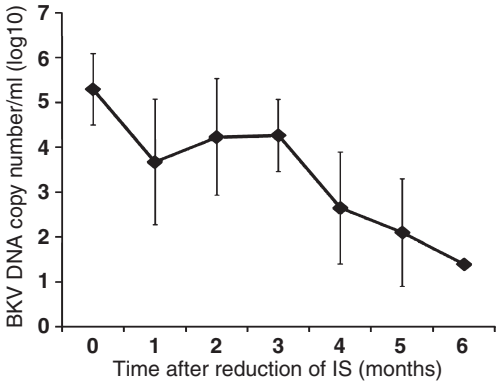

Figure 3 The change of plasma DNA copy number after the reduction of IS in patients with viremia. In the prospective cohort, significant BKV viremia ($>1 \times 10^{4}$ copies/ml) was detected in 12 patients. After reduction of immune suppressant, viremia was eliminated in 11 patients within 103 days (25–254). Data are expressed as mean ± sd after log-transformation.

Table 3. Evolution of renal function, viral load, and changes in IS in patients with presumptive BKVAN.

Patients	KT -initial viremia (day)	Initial viremia-clearance (day)	Scr (mg/dl)		Viral load*		Immune suppressants						
			At initial viral load	At last viral load	Initial	Last	Drug	Trough level (ng/ml)		MMF (mg)		Steroid (mg)	
								Before#	After#	Before	After	Before	After
1	124	121	1.48	1.22	4.76	0	Tac	9.3	5.2	1000	1000	10	5
2	273	25	1.20	1.19	4.0	0	Tac	4.5	5.1	1000	1000	5	5
3	91	161	1.40	1.27	4.53	0	Tac	5.4	4.9	1500	1000	10	10
4	84	30	0.95	1.32	5.08	0	Tac	7.3	7.2	1500	1500	10	10
5	287	40	1.05	0.96	6.00	0	Tac	5.7	4.5	1000	1000	5	5
6	49	37	1.46	1.56	5.86	0	Tac	7.1	7.2	1500	1500	10	10
7	109	182	1.38	1.07	6.69	0	Tac	5.1	4.9	1500	1500	10	10
8	58	254	1.30	1.33	5.97	0	Tac	12.9	5.7	1500	1500	10	10
9	105	–	1.29	0.93	5.73	4.5	Tac	6.2	–	1500	–	10	–
10	87	96	1.30	1.18	5.13	0	Tac	9.7	6.3	1500	1000	10	5
11	89	46	0.70	0.82	4.34	0	Tac	8.8	7.1	1500	1500	10	10
12	98	185	1.51	1.49	5.44	0	Tac	8.1	7.3	1500	1500	10	10

Tac, tacrolimus; MMF, mycophenolate mofetil.
*Numbers of copies were log-transformed.
#Before, before the development of viremia; after, after the clearance of viremia.

reduced at this time despite the sustained viremia. One month later, we performed a follow-up biopsy because of Scr elevation, and BKVAN stage A was diagnosed. MMF was subsequently discontinued, and the CNI dose was reduced. One month later, BKV viremia was eliminated and allograft function recovered as well.

Clinical progress of patients with and without viremia

Table 4 shows the comparison between the patients with and without viremia during the first year post-transplantation. Compared with those before transplantation, no significant differences were found in the clinical characteristics of the two groups, including donor type, use of desensitization protocol, and immunosuppressant type. The incidence of other opportunistic viral infections was slightly higher in the viremia group, but statistically insignificant as identified by Fisher's exact test. After reduction of immune suppressants, no cases of acute rejection developed in the viremia group. The frequency of acute rejection and change in the MDRD eGFR did not significantly differ during the first year post-transplantation between the two groups ($P > 0.05$, respectively). The trough level of Tac gradually decreased in both the groups; no significant difference was noted between the two groups ($P > 0.05$, at each time) (Fig. 4).

Table 4. Comparison between RTR with BKV viremia and without viremia.

	RTR with viremia ($N = 12$)	RTR without viremia ($N = 133$)	P
Follow-up (months)	20.9 ± 6.7	20.2 ± 6.0	0.73
Age (years)	43.9 ± 9.5	44.9 ± 10.9	0.75
Gender: male, *n* (%)	8 (66.7)	70 (52.6)	0.27
Retransplantation, *n* (%)	3 (25,0)	12 (9.2)	0.11
HLA mismatch number	3.3 ± 1.4	3.1 ± 1.8	0.68
ABO incompatibility, *n* (%)	1 (8.3)	8 (6.0)	0.38
Acute rejection, *n* (%)	1 (8.3)	19 (14.3)	0.48
Early rejection (<6 months)	1 (8.3)	2 (1.5)	
Late rejection (>6 months)	0 (0)	17 (12.8)	
Deceased donor, *n* (%)	41 (30.8)	6 (5.0)	0.15
Delayed graft function, *n* (%)	2 (16.7)	8 (6.0)	0.20
Opportunistic infection*, *n* (%)	2 (16.7)	3 (2.3)	0.06
Main Immune suppressant, *n* (%)			
Cyclosporine	0 (0.0)	13 (9.8)	0.39
Tacrolimus	12 (100.0)	120 (90.2)	
Desensitization before KT#, *n* (%)	2 (16.7)	10 (7.5)	0.26

RTR, renal transplant recipient; KT, kidney transplantation.
*RTR without viremia: one case of Parvovirus infection and one case of CMV viremia. RTR with viremia: three case of CMV viremia.
#Desensitization protocol composed of plasmapheresis, intravenous immune globulin, and rituximab.

Cost effectiveness of prospective plasma PCR monitoring

During the first year post-transplantation, a significantly higher frequency of BKV monitoring was performed in the viremia group (7.8 ± 1.7) compared with the nonviremia group (5.1 ± 0.5) ($P < 0.01$). The cost of plasma PCR per assay was US$45. Meanwhile, the cost for one 250-mg MMF tablet was US$0.5; therefore, when patients

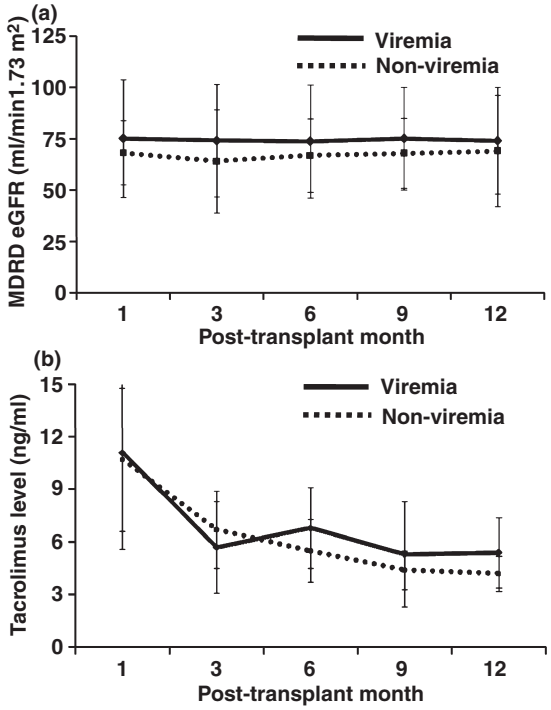

Figure 4 The comparison of allograft function and tacrolimus level between patients with viremia and without viremia. Note that (A) MDRD eGFR and (B) trough level of tacrolimus of patients with viremia during the first post-transplant year did not differ significantly from patients without viremia (vs. Nonviremia, $P > 0.05$, at each time).

consumed 1500 mg/day, the cost incurred was US$3/day. The discontinuation period of MMF in the viremia group was 103 days (25–254 days); therefore, the cost savings was about US$322 ± 224 per person. The increased cost for plasma PCR in the viremia group compared with the nonviremia group was US$165 ± 98 per person. Therefore, the cost savings as a result of IS reduction was significantly higher than the increased cost caused by additional plasma PCR in the viremia group ($P < 0.05$).

Discussion

In this study, plasma PCR demonstrated reliable diagnostic value, including high specificity and PPV, for the diagnosis of BKVAN. In addition, significant BKV viremia developed occasionally during the first year post-transplantation, but prospective IS reduction based on the plasma PCR results effectively eliminated BKV and prevented the development of BKVAN without increasing the risk of acute rejection.

We initially investigated the prevalence and clinical outcomes of BKVAN in our center. Regular monitoring of BKV replication is recommended when the prevalence of BKVAN exceeds 2.1% [10,24]. BKVAN developed in 3.7% of patients (14/376) in the retrospective cohort, which justified the need for prospective monitoring in our center. Of the 14 BKVAN cases, allograft failure developed in 28.6% (4/14); all of them were stage C, the most advanced stage. In the remaining 10 cases also, which were stage A or B, allograft function did not return to baseline value. All the above findings suggest that regular monitoring of BKV replication for the early detection of BKV infection is necessary in our center.

Various protocols for BKV monitoring have been developed and their effectiveness has already been demonstrated in several studies [4,16,24–28]. Some researchers recommend a step-wise approach, for example, the initial evaluation of viruria and quantitation of the BKV DNA load when the screening test is positive [4]. Buehring *et al.* [25] propose the use of surveillance biopsies to diagnose BKVAN. Another study used a urine PCR-based protocol and suggested sustained viruria as an early marker of significant BKV replication [26]. However, those protocols are not cost-effective and may be time consuming. In this regard, systematic and regular quantization of the plasma viral load by using BKV real-time PCR is proposed as an alternative approach in numerous studies [16,24,27,28].

However, neither the BKV real-time PCR method nor the cut-off value for significant viral replication has been standardized [15,19,21,22]. Therefore, previous protocols can have significantly different limits of quantization and dynamic ranges, leading to different conclusions regarding the cut-offs and predictive values of BKV viruria and viremia for BKVAN [18–20]. In this regard, we intended to formulate our center's own guidelines, including cut-off values for significant BKV replication in plasma PCR. In our retrospective cohort, cut-off values of 1×10^4 and 1×10^{10} copies/ml in plasma and urine PCR, respectively, showed the greatest sensitivity and specificity for the detection of BKVAN. Regarding diagnostic value, plasma PCR had superior PPV and specificity compared with urine cytology and urine PCR; this is concordant with the previous reports [2,21].

Therefore, we decided to perform regular monitoring of BKV replication using only plasma PCR and prospectively reduced IS according to the results of plasma PCR. In our prospective cohort, 12 cases of significant viremia were detected in the first year post-transplantation. As a result, viremia was eliminated successfully after IS reduction without the development of BKVAN in most of these patients; there was only one confirmed case of BKVAN (0.6%), which developed after treatment for acute rejection. In this patient also, immediate IS reduction abolished BKV replication and allograft function did not deteriorate. This suggests that early diagnosis and immediate reduction of IS can eliminate BKV and preserve

allograft function not only in viremia patients but also in confirmed BKVAN patients [23,29,30].

Some concern has been raised that tissue infiltration and allograft injury by BKV has already been initiated when significant viremia is detected, because it was regarded as a prerequisite for BKVAN [22,31]. During regular monitoring of plasma PCR, we performed allograft biopsy in seven patients who exhibited sustained viremia. We could not find any evidence of BKV involvement or tissue injury in the kidneys of those patients, and only acute CNI toxicity was found in all cases. In addition, after IS intensity was reduced, viremia was successfully eliminated in most patients, and allograft function remained stable. This suggests that the strategy of IS reduction after the detection of significant viremia could prevent the development of BKVAN and irreversible allograft injury.

The rate of BKVAN in the prospective cohort was significantly lower than that in the retrospective cohort (0.6% vs. 3.7%, respectively), although the intensity of IS was stronger in prospective cohort; for example, a higher proportion of patients used Tac and had ABO-incompatible KT or underwent the desensitization protocol before KT (Table 1). The PPV of plasma PCR for BKVAN was 54.5%, and the allograft failure rate in BKVAN was 28% (4/14) in the retrospective cohort. These results suggest that BKVAN and allograft failure would develop in about seven and two patients, respectively, of 12 viremia patients in the prospective cohort if regular monitoring was not performed. In the cost analysis during prospective monitoring, the cost savings caused by the withdrawal of MMF far exceeded the cost of performing additional plasma PCR in the viremia group; this suggests that there is no additional cost in the viremia group compared with the nonviremia group.

Positive plasma PCR indicates not only significant BKV replication, but also very strong IS intensity [32,33]. In this study, the prevalence of other opportunistic viral infections was higher in the viremia group than in the nonviremia group, although it did not reach statistical significance. In addition, CNI toxicity was detected in all allograft biopsy tissues from the viremia patients. The above findings justify IS reduction in these patients; consequently, acute rejection episodes may not increase after IS reduction.

A recent report recommends monitoring BKV replication every month during the first 6 months after KT [28]. It is also reported that monitoring every 3 months could not prevent BKVAN or allograft failure in some cases [4,27,28]. In contrast, we experienced only one case of BKVAN, which was successfully treated without allograft deterioration, with quarterly monitoring. This discrepancy may have resulted from the differences in the intensity of IS. In the above-mentioned reports, thymoglobulin was used as the induction therapy, and the dose of MMF was 2.0 g/day, which is stronger than that our study (basiliximab induction and 1.5 g/day MMF) [27,28]. There is consensus that the overall degree of IS is the most important risk factor for BKVAN [16,34–36]. Therefore, we thought that the monitoring interval could be determined according to the intensity of each center's IS regimen.

We performed BKV monitoring only up to 1 year post-transplantation because majority of BKVAN cases occur within the first year of transplantation [14,37]. This is because the IS regimen is usually strongest during this period. In this study, viremia appeared most frequently around 5 months after KT and cleared within 1 year. In the retrospective cohort, the interval from KT to the development of BKVAN was more than 2 years in most cases. However, considering that all cases were in advanced stage BKVAN with associated allograft dysfunction, it is possible that BKV replication and BKVAN had already developed within 1 year of KT.

This study has some limitations. We could not identify the risk factors for the development of BKV viremia. In previous reports, the use of Tac, steroid pulse therapy as a result of acute rejection, and induction by anti-thymocyte globulin were significantly associated with the development of viremia [38]. In this study, all viremia patients in prospective cohort took Tac, but the proportions of IS between the viremia and nonviremia patients was not significantly different, probably because of the limited sample size in this study. A prospective trial conducted in a larger cohort may be required to confirm the present results. Second, we did not investigate the effectiveness of each monitoring in the prospective cohort. A randomized controlled trial may be required for a more concrete conclusion regarding the superiority of plasma-PCR over other methods. Third, we only determine the clearance of BKV replication based on plasma PCR. Viruria and viremia have some correlation, but they also can develop separately according to the BKV replication kinetics [31]. Therefore, monitoring of viruia with viremia may be useful in determining of the complete clearance of viral replication.

In conclusion, there is an increased risk for BKVAN with positive plasma PCR, as viremia is likely to precede nephritis. In addition, regular monitoring using this method is useful for detecting the early stages of BKV replication. Prompt IS reduction according to the results prevents the development of BKVAN without increasing the risk of acute rejection. This strategy significantly prevents the development of BKVAN compared with the patients who do not undergo regular monitoring of BKV replication.

Authorship

BHC: participated in designing this study and writing the manuscript. YAH, HGK, and HSP: participated in collecting data. IOS, SRC, SHL, and YJC: participated in analyzing data. BSC, CWP, and Y-SK participated in performing study. CWY: participated in designing study.

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References

1. Hirsch HH. Polyomavirus BK nephropathy: a (re-)emerging complication in renal transplantation. *Am J Transplant* 2002; **2**: 25.
2. Costa C, Bergallo M, Astegiano S, *et al.* Monitoring of BK virus replication in the first year following renal transplantation. *Nephrol Dial Transplant* 2008; **23**: 3333.
3. Nickeleit V, Singh HK, Mihatsch MJ. Polyomavirus nephropathy: morphology, pathophysiology, and clinical management. *Curr Opin Nephrol Hypertens* 2003; **12**: 599.
4. Hirsch HH, Brennan DC, Drachenberg CB, *et al.* Polyomavirus-associated nephropathy in renal transplantation: interdisciplinary analyses and recommendations. *Transplantation* 2005; **79**: 1277.
5. Hurault de Ligny B, Etienne I, Francois A, *et al.* Polyomavirus-induced acute tubulo-interstitial nephritis in renal allograft recipients. *Transplant Proc.* 2000; **32**: 2760.
6. Kang YN, Han SM, Park KK, Jeon DS, Kim HC. BK virus infection in renal allograft recipients. *Transplant Proc.* 2003; **35**: 275.
7. Ahuja M, Cohen EP, Dayer AM, *et al.* Polyoma virus infection after renal transplantation. Use of immunostaining as a guide to diagnosis. *Transplantation* 2001; **71**: 896.
8. Barri YM, Ahmad I, Ketel BL, *et al.* Polyoma viral infection in renal transplantation: the role of immunosuppressive therapy. *Clin Transplant* 2001; **15**: 240.
9. Mengel M, Marwedel M, Radermacher J, *et al.* Incidence of polyomavirus-nephropathy in renal allografts: influence of modern immunosuppressive drugs. *Nephrol Dial Transplant* 2003; **18**: 1190.
10. Kiberd BA. Screening to prevent polyoma virus nephropathy: a medical decision analysis. *Am J Transplant* 2005; **5**: 2410.
11. Purighalla R, Shapiro R, McCauley J, Randhawa P. BK virus infection in a kidney allograft diagnosed by needle biopsy. *Am J Kidney Dis* 1995; **26**: 671.
12. Randhawa P, Brennan DC. BK virus infection in transplant recipients: an overview and update. *Am J Transplant* 2006; **6**: 2000.
13. Brennan DC, Agha I, Bohl DL, *et al.* Incidence of BK with tacrolimus versus cyclosporine and impact of preemptive immunosuppression reduction. *Am J Transplant* 2005; **5**: 582.
14. Hirsch HH, Knowles W, Dickenmann M, *et al.* Prospective study of polyomavirus type BK replication and nephropathy in renal-transplant recipients. *N Engl J Med* 2002; **347**: 488.
15. Bechert CJ, Schnadig VJ, Payne DA, Dong J. Monitoring of BK viral load in renal allograft recipients by real-time PCR assays. *Am J Clin Pathol* 2010; **133**: 242.
16. Wiseman AC. Polyomavirus nephropathy: a current perspective and clinical considerations. *Am J Kidney Dis* 2009; **54**: 131.
17. Randhawa P, Vats A, Shapiro R. Monitoring for polyomavirus BK And JC in urine: comparison of quantitative polymerase chain reaction with urine cytology. *Transplantation* 2005; **79**: 984.
18. Hirsch HH, Mohaupt M, Klimkait T. Prospective monitoring of BK virus load after discontinuing sirolimus treatment in a renal transplant patient with BK virus nephropathy. *J Infect Dis* 2001; **184**: 1494.
19. Hoffman NG, Cook L, Atienza EE, Limaye AP, Jerome KR. Marked variability of BK virus load measurement using quantitative real-time PCR among commonly used assays. *J Clin Microbiol* 2008; **46**: 2671.
20. Limaye AP, Jerome KR, Kuhr CS, *et al.* Quantitation of BK virus load in serum for the diagnosis of BK virus-associated nephropathy in renal transplant recipients. *J Infect Dis* 2001; **183**: 1669.
21. Viscount HB, Eid AJ, Espy MJ, *et al.* Polyomavirus polymerase chain reaction as a surrogate marker of polyomavirus-associated nephropathy. *Transplantation* 2007; **84**: 340.
22. Randhawa P, Ho A, Shapiro R, *et al.* Correlates of quantitative measurement of BK polyomavirus (BKV) DNA with clinical course of BKV infection in renal transplant patients. *J Clin Microbiol* 2004; **42**: 1176.
23. Drachenberg CB, Papadimitriou JC, Hirsch HH, *et al.* Histological patterns of polyomavirus nephropathy: correlation with graft outcome and viral load. *Am J Transplant* 2004; **4**: 2082.
24. Laskin BL, Goebel J. Cost-efficient screening for BK virus in pediatric kidney transplantation: a single-center experience and review of the literature. *Pediatr Transplant* 2010; **14**: 589.
25. Buehrig CK, Lager DJ, Stegall MD, *et al.* Influence of surveillance renal allograft biopsy on diagnosis and prognosis of polyomavirus-associated nephropathy. *Kidney Int* 2003; **64**: 665.
26. Babel N, Fendt J, Karaivanov S, *et al.* Sustained BK viruria as an early marker for the development of BKV-associated

nephropathy: analysis of 4128 urine and serum samples. *Transplantation* 2009; **88**: 89.
27. Almeras C, Foulongne V, Garrigue V, *et al.* Does reduction in immunosuppression in viremic patients prevent BK virus nephropathy in de novo renal transplant recipients? A prospective study. *Transplantation* 2008; **85**: 1099.
28. Almeras C, Vetromile F, Garrigue V, Szwarc I, Foulongne V, Mourad G. Monthly screening for BK viremia is an effective strategy to prevent BK virus nephropathy in renal transplant recipients. *Transpl Infect Dis.* 2011; **13**: 101.
29. Sener A, House AA, Jevnikar AM, *et al.* Intravenous immunoglobulin as a treatment for BK virus associated nephropathy: one-year follow-up of renal allograft recipients. *Transplantation* 2006; **81**: 117.
30. Schaub S, Hirsch HH, Dickenmann M, *et al.* Reducing immunosuppression preserves allograft function in presumptive and definitive polyomavirus-associated nephropathy. *Am J Transplant* 2010; **10**: 2615.
31. Funk GA, Gosert R, Comoli P, Ginevri F, Hirsch HH. Polyomavirus BK replication dynamics in vivo and in silico to predict cytopathology and viral clearance in kidney transplants. *Am J Transplant* 2008; **8**: 2368.
32. Koleilat I, Kushnir L, Gallichio M, Conti DJ. Initiation of a screening protocol for polyoma virus results in a decreased rate of opportunistic non-BK viral disease after renal transplantation. *Transpl Infect Dis.* 2011; **13**: 1.
33. Toyoda M, Puliyanda DP, Amet N, *et al.* Co-infection of polyomavirus-BK and cytomegalovirus in renal transplant recipients. *Transplantation* 2005; **80**: 198.
34. Smith JM, McDonald RA, Finn LS, Healey PJ, Davis CL, Limaye AP. Polyomavirus nephropathy in pediatric kidney transplant recipients. *Am J Transplant* 2004; **4**: 2109.
35. Ginevri F, De SantisR, Comoli P, *et al.* Polyomavirus BK infection in pediatric kidney-allograft recipients: a single-center analysis of incidence, risk factors, and novel therapeutic approaches. *Transplantation* 2003; **75**: 1266.
36. Awadalla Y, Randhawa P, Ruppert K, Zeevi A, Duquesnoy RJ. HLA mismatching increases the risk of BK virus nephropathy in renal transplant recipients. *Am J Transplant* 2004; **4**: 1691.
37. Hirsch HH, Steiger J. Polyomavirus BK. *Lancet Infect Dis.* 2003; **3**: 611.
38. Kotton CN, Fishman JA. Viral infection in the renal transplant recipient. *J Am Soc Nephrol* 2005; **16**: 1758.