Clinical and Immunological Characteristics for BK Polyomavirus-associated Nephropathy after Kidney Transplantation

Tanaya Siripoon

Mahidol University

Nopporn Apiwattanakul

Mahidol University

Pannawat Mongkolrattanakul

Mahidol University

Chutatip Tongsook

Mahidol University

Nattawut Unwanatham

Mahidol University

Suradej Hongeng

Mahidol University

Surasak Kantachuvesiri

Mahidol University

Jackrapong Bruminhent (✉ jbruminhent@gmail.com)

Mahidol University

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Abstract

Background

BK polyomavirus (BKPyV)-associated nephropathy (BKPyVAN) could cause allograft dysfunction among kidney transplant (KT) recipients. Intact BKPyV-specific immunity is correlated with viral containment, therefore clinical and immunological characteristics related to BKVAN after KT needs to be explored.

Methods

We prospectively investigated an association between clinical baseline characteristics, BK polyomavirus (BKPyV)-specific immunity, and BKPyV-associated nephropathy (BKPyVAN) in KT recipients. BKPyV-specific immunity, measured with intracellular cytokine assays, is reported as the percentage of IFN- γ -producing CD4⁺ T, CD8⁺ T, natural killer (NK), and NKT cells after large-T antigen and viral capsid protein 1 (VP1) stimulation.

Results

Among 100 KT patients, BKPyVAN occurred in 18% within one-year post-KT. There were 70 patients (70%) who received an induction immunosuppressive therapy. Diabetic nephropathy was significantly associated with BKPyVAN. Among 40 patients who were eligible for the immunological study. In a multivariate analysis, pre-KT %VP1-specific NK cells were independently associated with BKPyVAN (HR, 1.209; 95%CI, 1.055–1.386; P = 0.006). In those with BKPyVAN, the mean %NK (coefficient, 1.202; 95%CI, 0.033–2.371; P = 0.04), %VP1-specific NK (coefficient, 2.602; 95%CI, 1.083–4.121; P = 0.001), and %NKT (coefficient, 0.199; 95%CI, 0.051–0.348; P = 0.008) cells at 1-month post-KT were significantly higher than those pre-KT. Thus, increasing NK, VP1-specific NK, and NKT cell proportions were observed among KT recipients who developed BKPyVAN within the first year.

Conclusion

KT recipients with underlying diabetes and the presence of BKPyV-specific NK- and NKT-cell immunity could be at risk of BKPyVAN after KT. BKPyV-specific innate immune monitoring could potentially stratify those at risk of BKPyVAN among KT recipients. (Thai Clinical Trials Registry, TCTR20190404004)

1 Introduction

BK polyomavirus (BKPyV) infection is common among infectious complications after kidney transplantation (KT). Specifically, BKPyV-associated nephropathy (BKPyVAN) can result in allograft dysfunction, leading to unfavorable outcomes. Because KT recipients have defective immunity due to the immunosuppressants necessary to maintain their allograft, BKPyV can reactivate and manifest with

DNAuria initially, and it can progress to DNAemia then develop into BKPyVAN.¹ In addition to the direct effects of the virus, which can impair allograft function, decreasing a patient's immunosuppressive drug dose to contain the virus can potentially lead to acute allograft rejection.^{2–4} Since over-immunosuppression is a known risk factor for BKPyVAN in KT recipients; recent work has focused on restoring competent BKPyV-specific immune responses, which are a critical factor for controlling BKPyV infection, on stopping the active replication of this virus in KT recipients.^{3, 5, 6} A lack of either anti-BKPyV antibodies or BKPyV-specific T-cell responses before transplant is related to BKPyV reactivation after transplant.⁷ Previous studies have demonstrated that the presence of BKPyV-specific T cells, both prior to and early after transplant, is associated with virologic clearance in viremic patients.⁷ Although innate immunity components, such as natural killer (NK) and dendritic cells, also play a role in controlling BKPyV reactivation, the precise mechanism of their involvement is still unclear.⁸ Notably, a study on NK and NKT cell-specific immune responses in KT recipients who developed BKPyV infection after KT has not been much performed. Therefore, we conducted a prospective cohort study to investigate clinical, immunological factors, BKPyV-specific immune cell responses, and their association with BKPyVAN throughout this disease.

2 Materials And Methods

2.1 Study design

A prospective cohort study was conducted at a single transplant center in Bangkok, Thailand. All end-stage kidney disease (ESKD) patients aged 15 years or older who underwent KT from January to August 2019 were enrolled. We collected each patient's demographic data; transplant-related factors including allograft type, human leukocyte antigen (HLA) match, panel reactive antibodies (PRA), and immunosuppression type; clinical risk factors, immunologic risk factors; and clinical outcomes of BKPyV including DNAuria, viral DNA in the peripheral blood (DNAemia), and BKPyVAN. We preemptively measured urine and plasma BKPyV DNA loads prior to KT and at 1, 2, 3, 6, 9, and 12 months after KT (**Figure S1**). All patients with BKPyV viruria will be subsequently tested the collected plasma on the same date for viral DNA confirmed viremia. Additionally, we also performed a pilot study measured the percentages of CD4⁺ T, CD8⁺ T, NK, and NKT cells that produced interferon-gamma (IFN- γ) after stimulation with BKPyV-specific antigens prior to KT and at 1-month post-KT in subsets of the KT recipients.

Antimicrobial chemoprophylaxis, including trimethoprim/sulfamethoxazole for *Pneumocystis jirovecii*, acyclovir for herpes simplex virus, isoniazid for latent tuberculous infection, was offered to all patients. (Val) ganciclovir prophylaxis was offered for CMV-seronegative recipients who received an allograft from CMV-seropositive donor for six months and CMV-seropositive recipients who received anti-thymocyte globulin (ATG) induction therapy (3 months) after KT. The patients otherwise underwent preemptive CMV monitoring (3 months) post-KT.

The primary outcome of this study was to identify the incidence of BKPyVAN that occurred within one-year post-KT. The secondary outcomes were to assess clinical risk factors and outcomes of BKPyVAN. Finally, the exploratory outcome assessed the correlation between BKPyV-specific immunity and BKPyVAN within one-year post-KT. The Institutional Review Board of the Faculty of Medicine at Ramathibodi Hospital, Mahidol University, Bangkok, Thailand, approved the study protocol (approval No. ID 10-61-11). The study was conducted following the Good Clinical Practice Guidelines and the Declaration of Helsinki. All patients provided written informed consent before participation.

2.2 BKPyVAN

BKPyVAN was defined according to the guidelines recently published by the American Society of Transplantation Infectious Diseases Community of Practice (AST-IDCOP).^{3, 5} The various definitions of BKPyVAN included possible BKPyVAN (BKPyV DNAuria > 7 log₁₀ copies/mL), presumptive BKPyVAN (sustained BKPyV DNAemia > 3log₁₀ copies/mL within 3 weeks), probable BKPyVAN (BKPyV DNAemia > 4 log₁₀ copies/mL), and proven BKPyVAN (biopsy-proven polyomavirus-associated nephropathy (viral cytopathic change, inflammatory infiltrates or tubulitis or more than mild interstitial fibrosis/tubular atrophy). BKPyV DNAuria and DNAemia were defined as BKPyV DNA in the urine and plasma, respectively (**Table S1**). BKPyV DNA was detected and quantified with the Sentosa® SA, a BKPyV quantitative real-time PCR-based *in vitro* diagnostic test, using the Sentosa® SX101 instrument in conjunction with the Rotor-Gene® Q MDx 5plex HRM instrument (Vela Operations, Pte, Ltd., Singapore). Ethylenediaminetetraacetic acid-coated tubes were used for plasma collection along with urine sample collection were prepared with the reagents from the Sentosa® SX Virus Total Nucleic Acid Kit v2.0. The level of detection was 187 copies/mL (95% confidence interval (CI), 84.5–409.2), with a clinical sensitivity and specificity of 100% and 98.8%, respectively.

2.3 Intracellular BKPyV-specific immune responses

Laboratory-developed intracellular cytokine assays (ICAs) measuring the percentage of IFN- γ -producing CD4⁺ T cells, CD8⁺ T cells, NK, and NKT cells using LT and VP1 were performed before and at 1 month $\pm$ 7 days after transplant. The BKPyV-specific cells were the cells that are able to produce IFN- γ upon stimulation with LT or VP1. Both LT and VP1 antigens were accessed from JPT Peptide Technologies (Berlin, Germany). Peripheral blood mononuclear cells (PBMCs) were separated by density gradient techniques. Isolated PBMCs, prepared in RPMI1640 medium, were activated by specific peptides (LT or VP1: 1 μ g/mL). Activated cells by specific peptides and negative control stimulated cells were then further incubated at 37°C with 5% CO₂ for 18–24 hours. A blockade of cytokine secretion and intracellular accumulation was achieved by treatment with Brefeldin A (Biolegend, Inc., San Diego, CA, USA). At 3 hours after treatment with Brefeldin A, the cells were fixed with 2% formaldehyde (Sigma-Aldrich, Inc., St. Louise, MO, USA) and permeabilized with 0.1% Saponin (Sigma-Aldrich, Inc.) before being stained with fluorescent antibodies directed against the cytokine and finally analyzed by flow cytometry. The BKPyV-specific cell subset was identified by the direct immunofluorescence of the following monoclonal antibodies (eBioscience Inc. San Diego, CA, USA): anti-CD3 (labeled with FITC), anti-CD4 (labeled with

APC), anti-CD8 (labeled with APC efluor780), anti-CD56 (labeled with anti-cd56 labeled with PE) and anti-IFN- γ (labeled with PECy7). After being left to incubate with a cocktail of these antibodies for 30 minutes at 4°C in the dark, the cells were analyzed using FACSVerse (BD Pharmingen, Franklin Lakes, NJ, USA) and Flowjo software (Flowjo, LLC; Ashland, OR, USA). The results are expressed as the percentages of IFN- γ -producing CD4⁺, CD8⁺, NK, and NKT cells.

2.4 Statistical analyses

To estimate the incidence of BKPyVAN in this study, a sample size of 80 patients was calculated from a one-sample proportion test to detect 80% power. The BKPyVAN incidence of 20% reported by previous studies was used in this calculation^{2-4,9}. On the basis of an anticipated drop-off rate of 10%, we enrolled a total of 100 patients. The clinical characteristics of the patients were analyzed by descriptive analyses. Categorical and continuous data are described as absolute and relative frequencies and as means with standard deviation (SD), respectively. The cumulative incidence of BKPyVAN was estimated by applying the Kaplan-Meier methodology. Clinical and immunological factors for BKPyVAN (combined possible and presumptive BKPyVAN) were analyzed using the Cox proportional hazard model. The percentages of IFN- γ -producing CD4⁺ T, CD8⁺ T, NK, and NKT cells before and at 1-month post-KT were compared using a mixed-linear regression test. *P*-values of <0.05 were considered to indicate a statistically significant difference. Statistical analyses were conducted, and a linear plot of BKPyV-specific immune cells was constructed by using Stata statistical software (version 16, StataCorp, LLC, College Station, TX, USA).

3. Results

3.1 Baseline characteristics

Our study initially enrolled 100 KT recipients (Figure 1). Some patients were analyzed until censored after they died from an etiology other than BKPyVAN (*n* = 2), they had allograft loss not related to BKPyVAN (*n* = 5), or they were lost to follow-up (*n* = 3). The last date of follow-up was September 24, 2020. Clinical characteristics of the participants are shown in Table 1. Their mean age $\pm$ standard deviation (SD) was 42 $\pm$ 11 years. Sixty-five patients (65%) were males. The common end-stage kidney disease (ESKD) etiologies were: unknown etiologies (69%), glomerulonephritis (15%), and diabetic nephropathy (7%). Sixty-three (63%) patients underwent a deceased donor KT. Furthermore, 70% of the patients received induction immunosuppressive therapy, including basiliximab (67%) and antithymocyte globulin (ATG) (3%). They were later maintained on tacrolimus (81%), cyclosporin (19%), mycophenolate mofetil (56%), and prednisolone (99%). The average dose of mycophenolate mofetil was 1.4 grams per day. The average tacrolimus trough level was 4.9 ng/mL. Most cases carried cytomegalovirus (CMV)-seropositive donors and CMV-seropositive recipients (96%).

Table 1
Clinical characteristics of 100 kidney transplant recipients

Characteristics	N (%)
Female	35 (35)
Age (mean $\pm$ SD)	42 $\pm$ 11
ESKD etiologies (%)	
Diabetic nephropathy	7 (7)
Hypertension	5 (5)
Glomerulonephritis	15 (15)
Unknown	69 (69)
Others	4 (4)
Type of transplant	
DDKT	63 (63)
LRKT	37 (37)
BMI (mean $\pm$ SD)	22.4 $\pm$ 4.5
CMV serostatus (%)	
D+/R+	96 (96)
D-/R+	1 (1)
D+/R-	2 (2)
D-/R-	1 (1)
Terminal creatinine (median, IQR)	0.85 (0.52–2.51)
HLA mismatch (%)	
0	11 (11)
1–3	74 (74)
4–6	15 (15)
PRA (%)	
1–10	91 (91)
11–50	4 (4)
>50	5 (5)

Characteristics	N (%)
Induction therapy	
Basiliximab	67 (67)
Anti-thymocyte globulin	3 (3)
None	30 (30)
Maintenance therapy	
Tacrolimus	81 (81)
Cyclosporin	19 (19)
Mycophenolate sodium	43 (43)
Mycophenolate mofetil	56 (56)
Prednisolone	99 (99)

3.2 BKPyVAN

The cumulative incidence of BKPyVAN as estimated by applying the Kaplan-Meier method is shown in Figure 2 (A–B). The incidence of possible BKPyVAN and presumptive BKVAN is 17/100 (17%) and 1/100 (1%), respectively. Additionally, an incidence rate of 20.999/1000 and 1.166/1000 person-months, respectively. No proven BKPyVAN was identified in our cohort.

3.3 Clinical and immunological factors associated with BKPyVAN

Clinical and immunological factors associated with BKPyVAN were analyzed by the Cox proportional hazard model as shown in Table 2. In univariate analysis, underlying diabetic nephropathy (hazard ratio (HR), 7.548; 95% confidence interval (CI), 2.257–25.246; $P = 0.001$), underlying glomerulonephritis (HR, 3.355; 95%CI, 1.094–10.290; $P = 0.034$), pre-KT %VP1-specific NK cells (HR, 1.201; 95%CI, 1.058–1.363; $P = 0.005$), and 1-month post-KT %NKT (HR, 4.876; 95%CI, 1.482–16.050; $P = 0.009$) were significantly associated with BKPyVAN. In multivariate analysis, pre-KT %VP1-specific NK cells remained significantly associated with BKPyVAN (HR, 1.201; 95%CI, 1.058–1.363; $P = 0.005$).

Table 2

Univariate and multivariate analysis of clinical and immunological factors associated with BKPyVAN

Factors	Univariate analysis			Multivariate analysis		
	HR	95%CI	P-value	HR	95%CI	P-value
Female sex	0.958	0.354–2.594	0.933			
Age (per 1 year)	1.002	0.961–1.044	0.924			
Body mass index (per 1 kg/m ²)	0.998	0.896–1.111	0.967			
ESKD etiologies	7.548	2.257–25.246	0.001			
Diabetic nephropathy	3.355	1.094–10.290	0.034			
Glomerulonephritis	2.868	1.094–10.290	0.322			
Others	Ref.	0.356–23.098				
Unknown						
DDKT	1.195	0.448–3.186	0.722			
Creatinine on the day of discharge (per 1 mg/dL)	0.588	0.197–1.756	0.342			
HLA mismatch (per 1 mismatch)	1.094	0.424–2.823	0.852			
PRA (%)	3.647	0.829–16.070	0.087			
11–50	3.413	0.772–15.101	0.106			
>51	Ref.	15.101				
1–10						
Induction therapy	2.308	0.663–8.038	0.189			
Basiliximab	4.885	0.507–47.038	0.170			
Anti-thymocyte globulin	Ref.	47.038				
None						
Pre-KT BKPyV-specific immunity*						
%NK	1.127	0.989–1.284	0.073			
%LT-specific NK	0.860	0.527–1.404	0.546			

Factors	Univariate analysis			Multivariate analysis		
	HR	95%CI	<i>P</i> -value	HR	95%CI	<i>P</i> -value
%VP1-specific NK	1.201	1.058–1.363	0.005	1.209	1.055–1.386	0.006
%NKT	17.351	0.248–1215.659	0.188			
%LT-specific NKT	0.860	0.525–1.407	0.547			
%VP1-specific NKT	1.001	0.9889–1.126	0.993			
One-month BKPyV-specific immunity**						
%NK	1.033	0.775–1.378	0.824			
%LT-specific NK	1.097	0.562–2.145	0.785			
%VP1-specific NK	2.009	1.171–3.446	0.011			
%NKT	4.876	1.482–16.050	0.009			
%LT-specific NKT	0.700	0.275–1.781	0.454			
%VP1-specific NKT	0.898	0.603–1.338	0.598			

Intracellular flow cytometry analysis to determine IFN- γ production in BKPyV-specific immune responses in four representative KT recipients are shown in Figure 3 with BKPyV viremia (n=1) Figure 3.1, BKPyV viruria (n=1) Figure 3.2 and without BKPyVAN (n=2) **Figures 3.3 & 3.4.**

The results of a mixed-linear regression analysis of BKPyV-specific immunity associated with time among the 40 KT recipients whose BKPyV-specific immunity was assessed both prior to and 1-month after transplant are shown in **Table S2**. Among the participants with BKPyVAN, on average, the %VP1-specific NK (coefficient, 2.602; 95%CI, 1.083–4.121; *P* = 0.001), %NKT (coefficient, 0.199; 95%CI, 0.051–0.348; *P* = 0.008), and %NK (coefficient, 1.202; 95%CI, 0.033–2.371; *P* = 0.044) cells significantly increased over time from pre-KT to 1-month post-KT. A linear plot of those BKPyV-specific immune cells prior to and at 1 month after transplant in KT recipients who were diagnosed as free from BKPyVAN is shown in **Figure S2**.

3.5 Outcomes

At the 1-year follow-up, 27 (27%) patients had developed allograft dysfunction, and two patients (2%) had died of an undetermined cause unrelated to BKPyVAN. Additionally, five patients had experienced allograft loss, which was unrelated to BKPyVAN in four cases; the causes included intraoperative nephrectomy owing to immediate surgical complication (n = 1), emphysematous pyelonephritis of the transplanted allograft (n = 1), recurrent extended-spectrum beta-lactamase-producing *Escherichia coli* urinary tract infection complicated with bacteremia (n = 1), and *Pseudomonas aeruginosa*, and methicillin-resistant *Staphylococcus aureus* infected perinephric collection (n = 1). Seventeen patients with BKPyV viruria were subsequently tested for plasma BKPyV on the sample collected on the same date and dropped out of study for possible BKPyVAN treatment. Furthermore, one patient developed presumptive BKPyVAN and dropped out of the study for BKPyVAN treatment including calcineurin inhibitor reduction and corticosteroid tapering. The patient's immunosuppressive was switched to mTOR inhibitor afterward. Unfortunately, he later developed allograft rejection along with proven BKPyVAN, which caused allograft loss at 12 months post-KT.

4. Discussion

We performed clinical and pilot immunological studies evaluating non-specific and BKPyV-specific T-cell, NK-cell, and NKT-cell immune responses in KT recipients who developed BKPyVAN within the first year following KT. The present study identified individuals with BKPyV-specific innate immune responses prior to transplant, indicated by the presence of BKPyV-specific NK cells that secreted IFN- γ after stimulation with VP1 antigen along with the presence of diabetic kidney disease, as more likely to develop BKPyVAN within the first year post-KT. Additionally, the proportion of VP1-specific NK cells in KT recipients was observed to be slightly increased after transplant in accordance with BKPyVAN, despite post-transplant immunosuppression.

The demographic data of the participants in our study are comparable to those of previous studies, which reported the highest incidence of DNAemia as typically occurring within 3–6 months after transplantation.^{4,10,11} The previous two retrospective cohort studies conducted among Thai KT recipients demonstrated a BKPyVAN prevalence of 10–12%. However, this number could be an underestimate owing to the 50–70% compliance rate for post-transplant screening in our resource-limited setting.^{10,11} The incidence of possible BKPyVAN in the present study was relatively high (one out of five patients); however, the incidence of proven BKPyVAN was notably lower than that of the previous retrospective studies in a similar setting (6.4–8.6%), likely owing to our active monitoring for and early detection of BKPyV DNAuria and the consequent optimization of immunosuppression.^{10,11} Furthermore, underlying diabetes has been previously reported as a risk factor for BKPyVAN.³

Because an effective anti-BKPyV agent has not yet been established, vigilant immunosuppression adjustment in response to BKPyV-specific immune monitoring is crucial from the perspectives of both

prevention and treatment.^{1, 7, 12} However, the mechanisms of injury during BKPyV infection in KT patients are not completely understood. To address this gap, we conducted a prospective cohort study in which we assessed the immunological factors that contribute to early BKPyV infection after transplant.¹³ Viral-specific T-cell immunity plays an essential role in controlling viral infections in recipients of solid organ or hematopoietic stem cell transplants.¹³ The decline in viral-specific T-cell immunity has been shown to increase the risk of various infections, such as adenovirus, BKPyV, and CMV infections, in transplant recipients.^{13–16} While most studies have investigated the role of CMV-specific T-cell immunity and applied their findings in clinical practice via a commercial test, such as the QuantiFERON-CMV assay, there are limited studies on BKPyV-specific immunity^{17, 18}, which has left the mechanism of BKPyV persistence and reactivation unclear.⁸

To date, the cellular adaptive T-cell response has remained the most investigated and recognized aspect of controlling BKPyV infection; this response relies on the interplay between CD4⁺ and CD8⁺ T cells⁸. CD8⁺ T cells are predominantly specific to LT antigen, whereas CD4⁺ BKPyV-specific T cells mainly recognize VP1⁸. According to DeWolfe et al.⁷, in KT recipients who underwent BKPyV-specific T-cell immune monitoring along with preemptive BKPyV monitoring, low CD4⁺ and high CD8⁺ T-cell proportions and increased amounts of effector CD8⁺ T cells were found prior to transplant in patients who later developed DNAemia. Furthermore, increases in both CD4⁺ and CD8⁺ T cells were observed in patients with DNAemia who were able to achieve viral clearance after diagnosis⁷. Our group reported a marginal trend of LT-specific CD4⁺ T-cell restoration in the KT recipients who developed BKPyVAN after adjustment to their level of immunosuppression.¹⁴ Thus, a measurement of BKPyV-specific cellular responses might serve as a guide for fine-tuning the magnitude of immunosuppression to prevent BKPyV reactivation.

Although virus-specific T cells play a large role in the elimination of reactivated BKPyV, NK cells are early responders in the antiviral response. Our study demonstrated that the elevated pre-transplant BKPyV-specific innate immunity represented by a higher percentage of NK- and VP1-specific NK cells was independently associated with the development of BKPyVAN after transplant. The innate immune system consists of numerous cells and soluble molecules, and it plays essential roles in both suppressing viral replication and activating adaptive immunity to eradicate viruses.¹⁹ Inflammatory NK-cell antiviral responses involve interactions between activation receptors, such as killer-cell immunoglobulin-like receptors (KIRs), and host-cell MHC class I molecules, which alters cell sensitivity to lysis by NK cells.²⁰ After KT, the ischemic injury may reactivate BKPyV from the allograft kidney and trigger the innate immune system shortly after allograft reception. NK cells recognize virus-infected cells through the downregulation of MHC class I and inhibitory receptors as well as via the upregulation of activator molecules¹⁹. Patients with a small number of KIRs are at higher risk of developing BKPyVAN.^{8, 19} A detailed analysis of all receptors revealed significantly lower frequencies of the activating KIR in patients with BKPyVAN as compared with the controls²¹. Our findings support the potential role of NK cells in the pathogenesis of BKPyVAN development. Specifically, they reveal a correlation between the %VP1-specific NK cells and BKPyV infection. We demonstrated that patients with BKPyVAN had higher pre-KT %VP1-

specific NK and %NKT cells as compared with patients without high-level BKPyV DNAemia. Thus, innate immunity is important as the first line of defense against BKPyV infection. However, further studies and longer study periods are needed to address the associated mechanism.

We also investigated a cell type that reflects a bridge between the innate and adaptive immune systems, NKT cells. Unlike conventional T cells, which recognize peptide antigens presented by MHC molecules, NKT cells recognize glycolipid antigens presented by CD1d. Once activated, these cells can perform both functions commonly ascribed to T-helper cells and those characteristic of cytotoxic T cells. NKT cells have been reported to recognize and eliminate herpes viruses-infected cells.²² Gaya et al. showed that NKT cells also promote antibody responses during viral infection and can be detected up to three days before the formation of germinal centers in a mouse model. This NKT-cell role is believed to enhance B-cell antibody responses after viral infection.²³

There are limitations in this study. Higher anti-BKPyV titers have been reported to be related with post-transplant BKPyVAN because high donor BKPyV-specific antibody titers can be interpreted as a marker of recent viral exposure and a potentially higher BKPyV viral load in the allograft.^{24, 25} Unfortunately, we were unable to control for this because we did not have donor BKPyV serology. Additionally, we were able to measure BKPyV-specific immunity in only approximately half of the patients; the relatively small size of this group prevented us from exploring other associations. Larger studies regarding BKPyV-specific innate immunity is needed to confirm these data and their clinical implications. Because of our short follow-up period, we could not demonstrate long-term post-transplant outcomes and T-cell immunity. There were only a few patients in this study who developed BKPyV DNAemia and BKPyVAN, so the association between VP1-specific NK and NKT cells and presumptive and proven BKPyVAN may need a longer amount of time to be observed. Our team has continued to monitor the patients from this study for up to 2 years post-KT; this data collection is ongoing, and the result is eagerly anticipated. Last, the lack of a commercial assay for quantifying BKPyV-specific immunity could limit its use in some settings.

Notably, this study is the first work to demonstrate the clinical and immunological factors associated with BKPyV infection. Its findings emphasize the importance of a post-KT BKPyV screening surveillance system for KT recipients in light of the limited standardized treatment options. The 2019 guidelines from the AST-IDCOP suggest conducting screenings of the plasma BKPyV load or urine BKPyV viral load (optional) monthly until month 9 post-transplantation, followed by every 3 months until 2 years post-transplantation.³ Although urine monitoring is presented as optional in these newly updated guidelines, our study supports the use of such preemptive monitoring to prevent unfavorable consequences.

Since we included early stage of BKPyVAN (possible BKPyVAN), this allow us to explore BKPyV DNAemia which is a marker of early reactivation of BKPyV infection. Therefore, we were able to demonstrate early immune response such as innate immune response presented by BKPyV-specific NK cell responses. Our study also addresses the importance of both innate and adaptive BKPyV-specific immunity in KT recipients. Even the guideline from the AST-IDCOP does not recommend measuring either quantification or qualification of BKPyV-specific immunity prior to KT at the present time based on the fact that solid

evidence is still lacking to support the utilization. We believe our data would at least fulfill knowledge gap in this field³. Since there is a lack of evidence-based treatment options³, it is essential to closely monitor replication of BKPyV in KT recipients and promptly modify immunosuppressants in order to contain the virus to prevent further damage. The potential role of BKPyV-specific T-cell and antibody responses as adjunct markers to predict onset and clearance of BKPyV DNAemia needs to be defined, since this information can guide the reduction in immunosuppressants and the need for re-increase after clearance. Further research is needed to establish mechanisms that contribute to BKPyV infection and the affected pathways which are critical for immune defense against other pathogens.

5. Conclusion

Our study shows a significant trend of greater initial levels and post-KT increases in NK, NKT, and VP1-specific NK cells among KT recipients who developed BKPyVAN with 1-year post-KT. BKPyV-specific NK- and NKT-cell immune monitoring could potentially be used to identify patients at risk of BKPyV reactivation after transplant. Further studies are required to confirm this correlation, determine the underlying mechanisms and explore clinical relevance in practice.

Abbreviations

ALC, Absolute lymphocyte count

AST-IDCOP, American Society of Transplantation Infectious Diseases Community of Practice

ATG, Anti-thymocyte globulin

BKPyV, BK polyomavirus

BKPyVAN, BK polyomavirus-associated nephropathy

BMI, Body mass index

CD1d, Clusters of differentiation 1d

CD4, Clusters of differentiation 4

CD8, Clusters of differentiation 8

CI, Confidence interval

CMV, Cytomegalovirus

CO₂, Carbon dioxide

D, Donor

DDKT, Deceased-donor kidney transplantation

DNA, Deoxyribonucleic acid

DNAemia, Viral DNA in the peripheral blood

DNAuria, Viral DNA in the urine

ESKD, End-stage kidney disease

HLA, Human leukocyte antigen matching

HR, Hazard ratio

ICA, Intracellular cytokine assay

IFN- γ , Interferon-gamma

KIR, Killer-cell immunoglobulin-like receptor

KT, Kidney transplantation

LRKT, Living-related kidney transplantation

LT, Large-T

MHC, Major histocompatibility complex

Mg, Milligram

mL, Milliliter

NK, Natural killer

NKT, Natural killer T

PBMC, Peripheral blood mononuclear cell

PCR, Polymerase chain reaction

PRA, Panel-reactive antibody

R, Recipient

Ref., Reference

SD, Standard deviation

Declarations

Ethics approval and consent to participate: The protocol for this study was approved by the Institutional Review Board of the Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand with the provisions of the Good Clinical Practice Guidelines and the Declaration of Helsinki. All patients provided written informed consent prior to participation.

Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary information files.

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3. Participated in the performance of the research: TS, PM, CT, JB
4. Contributed new reagents or analytic tools: NA, CT, SH
5. Participated in data analysis: TS, NU, JB

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Figures

Figure 1

Flow chart of the study.

Figure 2

Figure 2

Cumulative incidence of possible or presumptive BKPyV-associated nephropathy. (A–B) Kaplan-Meier plot of cumulative (A) or presumptive (B) BKPyV-associated nephropathy within one-year post-KT.

Figure 3.1 (A-E) Patient 004 (presumptive BKPyVAN)

Figure 3.2 (A-E) Patient 043 (possible BKPyVAN)

Figure 3.3 (A-E) Patient 061 (no BKPyVAN)

Figure 3.4 (A-E) Patient 076 (no BKPyVAN)

Figure 3

Analysis of laboratory-developed intracellular cytokine assays (ICAs) measuring the percentage of IFN- γ -producing CD4⁺ T cells, CD8⁺ T cells, NK, and NKT cells after incubating with LT and VP1. Figure 3.1 (A-E) represent patient 004 (presumptive BKPyVAN). Figure 3.2 (A-E) represent patient 043 (possible BKPyVAN). Figure 3.3 (A-E) represent patient 061 (no BKPyVAN). Figure 3.4 (A-E) represent BK patient 076 (no BKPyVAN). PBMCs were incubated in RPMI 1640 with 5% CO₂ for 18-24 hours at 37°C. Isolated PBMCs were activated by specific peptides (LT or VP1). (A) Demonstrate gating strategy of negative control [side scatter (SSC) vs CD56 (left panel) and CD3 vs CD56 (right panel)] for identification of CD3⁻ and CD3⁺ CD56⁺ NK cells. (B) Demonstrate IFN- γ -producing CD3⁺CD56⁺ and CD3⁻CD56⁺ cells after stimulation by LT or VP1. Gating strategy of side scatter (SSC) vs CD3⁺ (left panel), CD4⁺ vs CD8⁺ (middle panel), and

CD4+ and CD8+ producing IFN- γ (right panel)] in negative control (C), after stimulation by VP1 (D) or after stimulation by LT (E)

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