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# A Preliminary Study Into the Significance of Intrarenal Reflux in BK Virus Nephropathy After Kidney Transplantation

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**Background.** The BK virus typically colonizes the lower urinary tract and is the causative agent in BK virus nephropathy (BKVN), which can progress to allograft dysfunction and graft loss. Urinary reflux in kidney allografts is induced by vesicoureteral reflux or disturbances in intrarenal reflux (IRR), believed to be associated with BKVN. This study was designed to elucidate the relationship between BKVN and IRR. **Methods.** We examined 30 renal transplant recipients histologically diagnosed with BKVN using anti-Simian virus 40 immunohistochemistry and 60 clinically matched control recipients. The BKVN patients were divided into stable (n = 12) and progressive (n = 18) groups according to allograft kidney function 1 year after diagnosis. Histological rejection scores according to the pathological classification of rejection in renal allografts (Banff classification), histological BKVN stages, and histological polyomavirus load levels (pvl) proposed by the Banff working group were evaluated. The IRR was quantified by histological reflux scores defined with retention and reflux of immunostained Tamm-Horsfall protein in renal tubules and glomeruli. **Results.** Higher reflux scores were observed in the BKVN group compared with that in the control group. No differences in clinical parameters were observed between the BKVN and control groups. Reflux scores and pvl were significantly higher in the progressive group than in the stable BKVN group with no significant difference in BK stage observed between groups. Reflux scores were found to be significantly correlated with pvl. **Conclusions.** Our preliminary study suggested that IRR might be a predisposing and prognostic factor in BKVN.

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Tubulointerstitial nephritis caused by the BK virus and subsequent interstitial fibrosis, termed BK virus nephropathy (BKVN) or polyomavirus-associated nephropathy occurs in 1% to 10%<sup>1</sup> of kidney transplant recipients. The BKVN may progress to allograft dysfunction or loss in 15% to 50%<sup>2,3</sup> of infected individuals. Primary BK virus infections occur early in childhood via oral and/or respiratory exposure and the virus remains latent in the renal epithelium (transitional epithelium, renal tubular epithelium, and parietal epithelium of Bowman capsule) and lymphoid cells.<sup>4</sup> The

degree of immunosuppression is likely the most important risk factor underlying BK viral infection, with BK replication considered a reliable marker of excess immunosuppression.<sup>5</sup>

However, the susceptibility of allograft kidneys to other forms of injury, such as ischemia and rejection, may explain why majority of the cases of BKVN occur after renal transplantation, and only rarely occur after liver, heart, lung, or bone marrow transplantation.<sup>6</sup>

Several important studies have established effective strategies for the management of BKVN after kidney transplantation,<sup>7-10</sup> particularly at the timing of increasing immunosuppression: screening and preemptive monitoring of viremia using cytology

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The name of the trial registry: The investigation for association between BK virus nephropathy and reflux nephropathy in a retrospective study.

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(decoy cells), measurement of urinary DNA loads or urinary VP-1 mRNA loads, and measurement of viremia using plasma DNA loads.

However, allograft biopsies are still required to confirm the diagnosis of BKVN and assess for other histological types of injury, particularly concurrent acute rejection.

The BKVN is histologically diagnosed using anti-Simian virus 40 (SV40) immunohistochemistry with disease stages (A, B, and C) and histological polyomavirus load levels (pvl) proposed by the Banff working group. The BKVN disease stages (particularly stage C) and pvl have been shown to correspond with allograft outcomes.<sup>11</sup> However, the distinction of BKVN from acute tubular necrosis, interstitial nephritis, and acute cellular rejection remains challenging.<sup>12</sup>

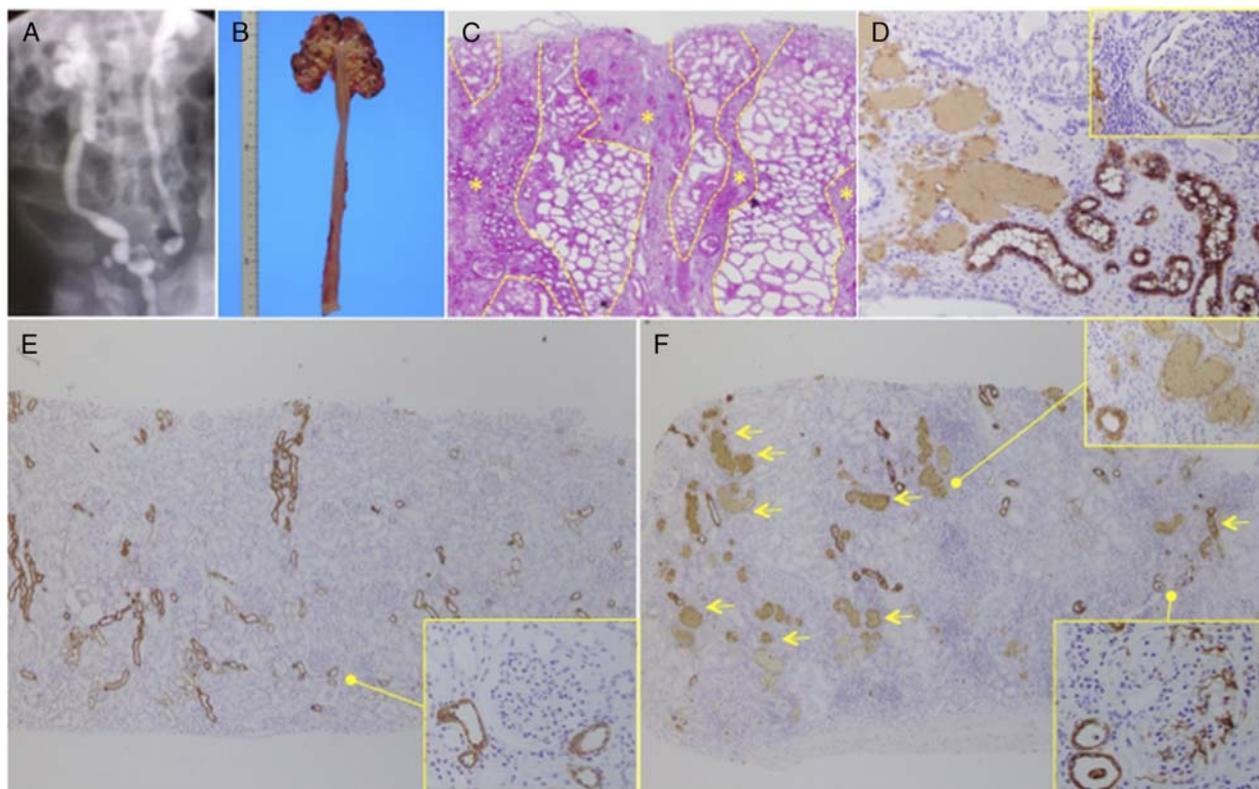
On the other hand, an increased incidence of BK viremia has been associated with urinary stenosis and the use of ureteral stents.<sup>13-15</sup> These reports suggest that urinary reflux in kidney allografts is induced by vesicoureteral reflux (VUR) or intrarenal reflux (IRR), believed to be associated with BKVN.

The VUR is commonly diagnosed using a voiding cystourethrogram (VUCG), according to the classification of the International Reflux Study group (grades 1-4)<sup>16</sup> and involves the catheterization of the bladder and radiopharmaceutical imaging of the lower and upper urinary tract (Figure 1A). Histologically reflux nephropathy associated with VUR or IRR is characterized by acquired focal renal scarring with interstitial mononuclear cell infiltration and fibrosis, particularly in

medullary rays comprising proximal and distal tubules going to and from the medulla and collecting ducts (Figures 1B and C), which is termed medullary ray injury (MRI) by Kobayashi et al.<sup>17</sup> The etiologies of MRI are considered to be ischemic and urological complications, such as atherosclerosis, calcineurin inhibitor (CNI)-associated vasculopathy, urinary tract infection (UTI), and urinary obstruction or reflux.<sup>17</sup>

Reflux nephropathy is also associated with interstitial Tamm-Horsfall protein (THP) deposition or THP reflux into the Bowman capsule (Figures 1D and F) due to combined VUR and IRR associated with UTI, or permanently high intravesical pressures in human VUR patients<sup>18,19</sup> or animal reflux nephropathy models.<sup>20</sup> In kidney transplant settings, Kobayashi et al<sup>17</sup> reported that a urinary obstruction-related MRI (THP-positive cast occlusive type) was found in 85.7% kidney recipients with a diagnosis of VUR (grades 1-3) based on VUCG. Akioka et al<sup>21</sup> also reported interstitial THP deposition in 26.3% pediatric recipients, with 80% diagnosed with VUR (grades 1-4) based on VUCG. Thus, THP staining is believed to have utility in detecting VUR or IRR in kidney biopsy samples, particularly in kidney transplant settings.

The THP, also known as uromodulin, is a glycoprotein with a variety of N-linked and O-linked glycans that confer the ability to bind a wide range of substances.<sup>22,23</sup> Further, THP has been shown to perform a defensive role against UTI, presumably by contributing to the binding and clearance of uropathogenic bacteria from the urinary tract.<sup>24,25</sup> Recently, the presence of 3-dimensional THP-rich viral clusters,



**FIGURE 1.** Representative image of reflux nephropathy. A, Diagnosis of VUR with a VUCG according to the classification of the International Reflux Study Group (grade 4). B, Macroimage of nephrectomy for recurrent VUR. C, Microscopic image of reflux nephropathy demonstrating medullary ray injury (yellow asterisk) according to the presence of PAS-stained casts. D, The THP immunostaining revealed THP occlusion in tubuli, deposition into the interstitium, and THP reflux into the Bowman space. E, Living donor kidney at the kidney transplantation (0-hour biopsy) demonstrated no THP obstruction of the tubuli or glomerular deposition. F, Reflux nephropathy kidney due to severe urinary stenosis at the renal autotransplantation (0-hour biopsy) demonstrating severe occlusions with THP casts in tubuli (yellow arrow) and THP deposition in glomeruli. ([c] PAS stain; [d, e, f] THP immunohistochemistry). PAS, Periodic acid-Schiff;

termed “Haufen,” have been shown to be a reliable marker of BKVN in viremic or persistently viruric patients with negative histological findings on renal biopsy.<sup>26</sup> It is considered that “Haufen” are formed during cellular rupture and release of daughter virions into injured nephrons, with high levels of THP shown to aggregate as well as the influenza virus.<sup>27</sup> The THP may perform both proinflammatory and antiviral roles in BKVN; however, we hypothesize that THP backflow into the tubulointerstitium and Bowman capsule occurs due to urinary reflux, which may lead to dispersion of BK virions into kidney allografts.

In the present study, we preliminarily evaluated the association between BKVN and urinary reflux in kidney allografts induced by VUR or disturbances in IRR. The IRR was systematically evaluated using THP immunohistochemistry in renal biopsy samples from 30 renal transplant recipients with BKVN and 60 control recipients.

## MATERIALS AND METHODS

### Patients and Allograft Biopsies

We performed a retrospective analysis of 90 kidney transplant recipients who underwent allograft kidney biopsy at our center between April 2003 and March 2015. The study was approved by the local ethical committee (approval no 3367). Cases were retrieved over a 12-year period from the pathological records of the kidney center at the Tokyo Woman's Medical University. We identified 60 control renal allograft recipients matched according to age and posttransplantation period. The BK virus plasma DNA loads (threshold;  $>10^4$  copies/mL) were available in 21 BKVN patients. All clinical data were retrieved from patient medical records. For the histological study, 164 kidney allograft biopsies from 30 patients with BKVN were evaluated, including preceding biopsies ( $n = 29$ ), SV40-proven biopsies ( $n = 49$ ), SV40-cleared biopsies ( $n = 26$ ), and controls ( $n = 60$ ).

### Morphometric Analysis

Tissue was processed after formalin fixation using standard histological techniques. Frozen sections were prepared for immunofluorescence. Histological rejection scores according to the Banff classification 2013,<sup>28</sup> histological BKVN stages, and pvl proposed by the Banff working group<sup>11</sup> were independently scored by 3 pathologists. The BKVN diagnoses were confirmed by SV40 immunohistochemistry (mouse monoclonal IgG2a, Calbiochem, Merck KGaA, Darmstadt, Germany). Histological BK stages and 3-tier pvl scores (score 1, 1%; score 2, 1–10%; score 3,  $>10\%$  according to the overall percentage of SV40-positive tubules) by the Banff working group<sup>11</sup> were evaluated.

The THP immunohistochemistry using an antihuman THP antibody (mouse monoclonal IgG, Cedarlane, Burlington, ON, Canada) was performed to evaluate renal reflux with a scoring system used assessing each tubule and glomerulus in nonatrophied cortical areas. In brief, scores were assigned for (1) tubular involvement: score 0, normal THP staining in the medullary ray area, particularly the thick ascending limbs of the loop of Henle (Figure 1E); score 1, tubular luminal occlusion with THP-positive casts observed in 10% to 25% of the medullary ray lesion area; score 2, 25% to 50%; and score 3, greater than 50% and (2) glomerular involvement: score 0, no THP staining; score 1, THP reflux

affecting 1% to 10% of the glomerular capsular space; score 2, 10% to 25%; and score 3, greater than 25% of the glomerular capsular space (Figure 1F). Histological reflux scores were calculated as the average of both tubular and glomerular components.

### Data and Statistical Analyses

Clinical data were analyzed between 30 BKVN patients and 60 control renal allograft recipients. A separate analysis was subsequently performed for stable ( $n = 12$ ) and progressive ( $n = 18$ ) BKVN, defined according to the absence or presence of increased serum creatinine levels of 0.5 mg/dL or greater, respectively, over a 1-year period from initial SV40-proven diagnoses of BKVN. A total of 104 allograft biopsies from BKVN patients were evaluated including preceding biopsies ( $n = 29$ ), SV40-proven biopsies ( $n = 49$ ), SV40-cleared biopsies ( $n = 26$ ), and 60 biopsies from control recipients. JMP Pro 11.2.0 (SAS Institute Inc, SAS Campus Drive, Cary, NC) was used for statistical analyses. Prism 6 (GraphPad Software, Inc, La Jolla, CA) was used for graph preparation. Data were presented as medians (range). Multiple comparisons were performed using the Kruskal-Wallis test followed by the Mann-Whitney test. Correlations were assessed using the nonparametric Spearman rank correlation coefficient  $\rho$ . Continuous variables were compared using the Mann-Whitney test and categorical variables were compared using Fisher exact test. The pvl scores were compared using the Jonckheere-Terpstra exact test for trend in SAS 9.4 TS 1M1 (SAS Institute Inc, SAS Campus Drive Cary, NC). In all analyses,  $P$  values less than 0.05 were considered statistically significant.

## RESULTS

### Clinical Background

Clinical characteristics of BKVN ( $n = 30$ ) and non-BKVN ( $n = 60$ ) patients are shown in Table 1. Primary kidney diseases, except diabetic and congenital anomalies of kidney and urinary tract, were as follows: glomerulonephritis in 31 (34.4%) patients; other genetic kidney diseases, such as Alport syndrome, autosomal dominant polycystic kidney disease, and nephronophthisis in 4 (4.4%) patients; and other causes, such as hypertension or undefined, in 35 (38.9%) patients. No differences in clinical characteristics were observed between control recipients and BKVN patients (Table 1). In the BKVN group, ureteral obstruction was observed in 6 recipients (20%), with 3 patients requiring periodical replacement of ureteral stents. All BKVN patients were treated with reduced immunosuppression regimens, with cidofovir additionally prescribed in 3 patients (10%). Graft loss was observed in 5 (16.7%) BKVN patients due to persistent BKVN ( $n = 3$ , 10%) or combination of BKVN and rejection ( $n = 2$ , 6.7%). Two patients were found to have simultaneous acute/active AMR and 1 patient progressed to chronic, active AMR. A greater postoperative duration was observed in the progressive group compared to the stable group; however, no differences were observed in other clinical parameters, including BK viremia (Table 2). No patients died during the observation period.

### Histological Characteristics of BKVN

All BKVN patients were histologically diagnosed with immunohistological staining for the SV40 antigen (Figures 2A and B). Reflux nephropathy was diagnosed according to the

**TABLE 1.**  
Clinical characteristics of the patients in the study

	BKVN (n = 30)	Control (n = 60)	P
Age, y	48.2 (3-66)	44.6 (6-71)	0.16
Sex (male %)	22 (73.3%)	37 (61.7%)	0.35
Postoperative day at biopsies <sup>a,b</sup>	306 (63-948)	358 (10-1565)	0.56
Donor age, y	60 (18-74)	55.3 ± 1.5	0.28
Deceased donor	6 (20.0%)	10 (16.7%)	0.77
ABO incompatible	6 (20.0%)	10 (16.7%)	0.77
No. mismatched HLA (A, B, DR)	3 (0-6)	3 (0-6)	0.88
No. CAKUT	1 (3.3%)	6 (10.0%)	0.42
Vesicoureteral reflux	6 (20.0%)	9 (15.0%)	0.56
Diabetes	7 (23.3%)	8 (13.3%)	0.24
Previous history of rejection	7 (23.3%)	16 (26.7%)	0.80
Previous history of CMV infection	12 (40.0%)	13 (21.7%)	0.0832
Serum creatinine at baseline (mg/dL)	1.36 (0.4-2.9)	1.55 (0.34-4.36)	0.43
Basiliximab	21 (70.0%)	47 (78.3%)	0.43
Rituximab	15 (50.0%)	29 (48.3%)	1.00
Plasma apheresis	5 (16.7%)	19 (31.7%)	0.14
Others <sup>c</sup>	4 (13.3%)	2 (3.3%)	0.17
Tacrolimus and mycophenolic acid	26 (86.7%)	48 (80.0%)	0.56
Cyclosporine and mycophenolic acid	4 (13.3%)	10 (16.7%)	0.77

Data were described by median (range). Categorical variables were compared by Fisher exact test and continuous variables were compared by Mann-Whitney test. All patients were maintained on prednisone from time of transplant.

<sup>a</sup> BKVN was proven with SV40 immunostaining for the first time (n = 30).

<sup>b</sup> The control group (n = 60) comprised surveillance biopsies (n = 40) and diagnostic biopsies for allograft dysfunction (n = 15) and abnormal urinalysis (n = 5).

<sup>c</sup> Included everolimus or azathioprine in addition to CNI or other immunosuppressive drug.

CAKUT, congenital anomalies of kidney and urinary tract; CMV, cytomegalovirus.

detection of THP in tubules and glomeruli by immunohistochemical staining (Figures 2C, D, and E). In the BKVN group, 11 patients had stage A disease, 17 patients had stage B disease, and the remaining 2 patients had stage C disease. The pvl scores according to the criteria proposed by Banff working group were 1, 2, and 3 in 8, 13, and 9 patients, respectively, at initial diagnosis before treatment. From the point of view of T cell-mediated rejection (TCMR) and antibody-mediated rejection (AMR), borderline changes, TCMR, AMR, and mixed TCMR and AMR were observed in 1, 2, 2, and 2 patients in the BKVN group, respectively. On the other hand, borderline changes, TCMR, AMR, and mixed type rejection, were observed in 3, 3, 2, and 1 patients in the control group. No significant difference in Banff scores was observed in preceding biopsies (SV40-negative) between the BKVN group (n = 29) and controls (n = 60, Figures 3A-C). On the other hand, reflux scores in preceding biopsies were higher in the BKVN group compared with the control group (Figures 3A and D). Histological reflux scores were highest in SV40-proven biopsies compared with that in all other biopsy types in the BKVN group (Figures 3A and D). Moreover, reflux scores were higher in the progressive BKVN group than in the stable group, with no difference in Banff scores observed between groups (Figure 3B).

### Histological Prognostic Factor in BKVN

The pvl scores at initial histological diagnosis correlated completely with BK viremia (Figure 4A); however, pvl scores were significantly higher in progressive BKVN patients than in stable BKVN patients using the Jonckheere-Terpstra exact test for trend ( $P = 0.0071$ ). A significant correlation was observed between pvl scores and  $t$  scores, which reflect BK staging according to Banff criteria and reflux scores (Figures 4B and D). Reflux scores did not correlate with either  $t$  scores

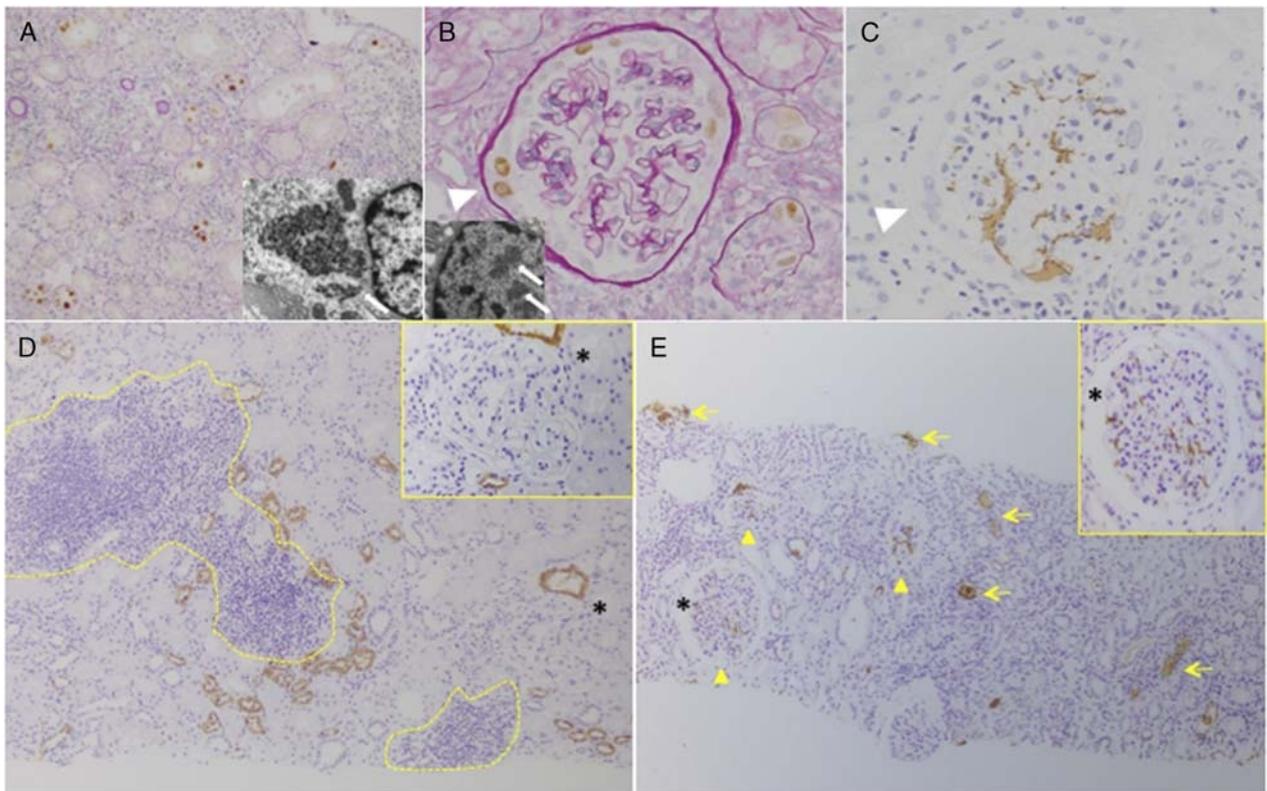
**TABLE 2.**  
Clinical characteristics of the stable and progressive BKVN patients

	Stable (n = 12)	Progressive (n = 18)	P
Age, y	50 (3-67)	47 (29-66)	0.85
Sex (male %)	8 (66.7%)	14 (77.8%)	0.68
Postoperative day at biopsies <sup>a</sup>	170 (63-947)	415 (91-948)	0.021
Donor age, y	53 (18-73)	64 (40-74)	0.0793
Deceased donor	3 (25%)	3 (16.7%)	0.66
ABO incompatible	2 (16.7%)	4 (22.2%)	0.36
No. mismatched HLA (A, B, DR)	3 (1-5)	3 (1-6)	0.25
Vesicoureteral reflux	2 (16.7%)	4 (22.2%)	1
Diabetes	3 (25%)	4 (22.2%)	1
Previous history of rejection	3 (25%)	4 (22.2%)	1
De novo DSA by the Luminex method	0/4 (0%)	1/12 (8.3%)	1
Previous history of CMV infection	5 (41.7%)	7 (38.9%)	1
Serum creatinine at baseline (mg/dL)	1.37 (0.4-2.9)	1.36 (0.74-2.5)	0.70
Serum creatinine at biopsies (mg/dL) <sup>a</sup>	1.78 (0.53-3.8)	1.98 (1.12-5.51)	0.27
Basiliximab	8 (66.7%)	13 (72.2%)	1
Rituximab	4 (33.3%)	11 (61.1%)	0.26
Plasma apheresis	1 (8.3%)	4 (22.2%)	0.62
Tacrolimus and mycophenolic acid	10 (83.3%)	16 (88.9%)	1
Virus DNA in plasma ( $10^4$ copies/mL)	0.46 (0-9.7)	4.3 (0-230)	0.23
BK viremia ( $> 10^4$ copies/mL)	2/6 (33.3%)	8/15 (53.3%)	0.64

Data were described by median (range). Categorical variables were compared by Fisher exact test and continuous variables were compared by Mann-Whitney Test.

<sup>a</sup> BKVN was proven with SV40 immunostaining for the first time.

DSA, donor-specific antigen



**FIGURE 2.** Representative BK polyoma virus nephropathy and micro urinary reflux. A, Severe tubulointerstitial nephritis with epithelial cell lysis and denudation of the TBM (BK stage B). Tubular epithelial cells were diffusely positive for SV40 staining (pvl score, 3). TEM image demonstrating BK virus particles within tubular epithelial cells ( $\times 15\,000$ , white arrow). B, Parietal epithelial cells lining Bowman capsule demonstrated evidence of viral replication and virus particles were detected on TEM images ( $\times 20\,000$ , white arrow). C, Urinary reflux demonstrated by THP staining in serial sections (white triangle showed same lesion). D, Transplant kidney biopsy in the control group demonstrating no THP obstruction in tubuli or deposition in glomeruli (asterisk showed same lesion) despite the presence of tubulointerstitial rejection (yellow dots). E, Reflux nephropathy detected in the BKNV group demonstrated THP cast occlusion in tubuli (yellow arrow) and THP deposition in glomeruli (yellow triangle, asterisk showed same lesion). [a, b] SV40 immunohistochemistry with PAS stain and TEM images, [c, d, e] THP immunohistochemistry; TEM, transmission electron microscopy;

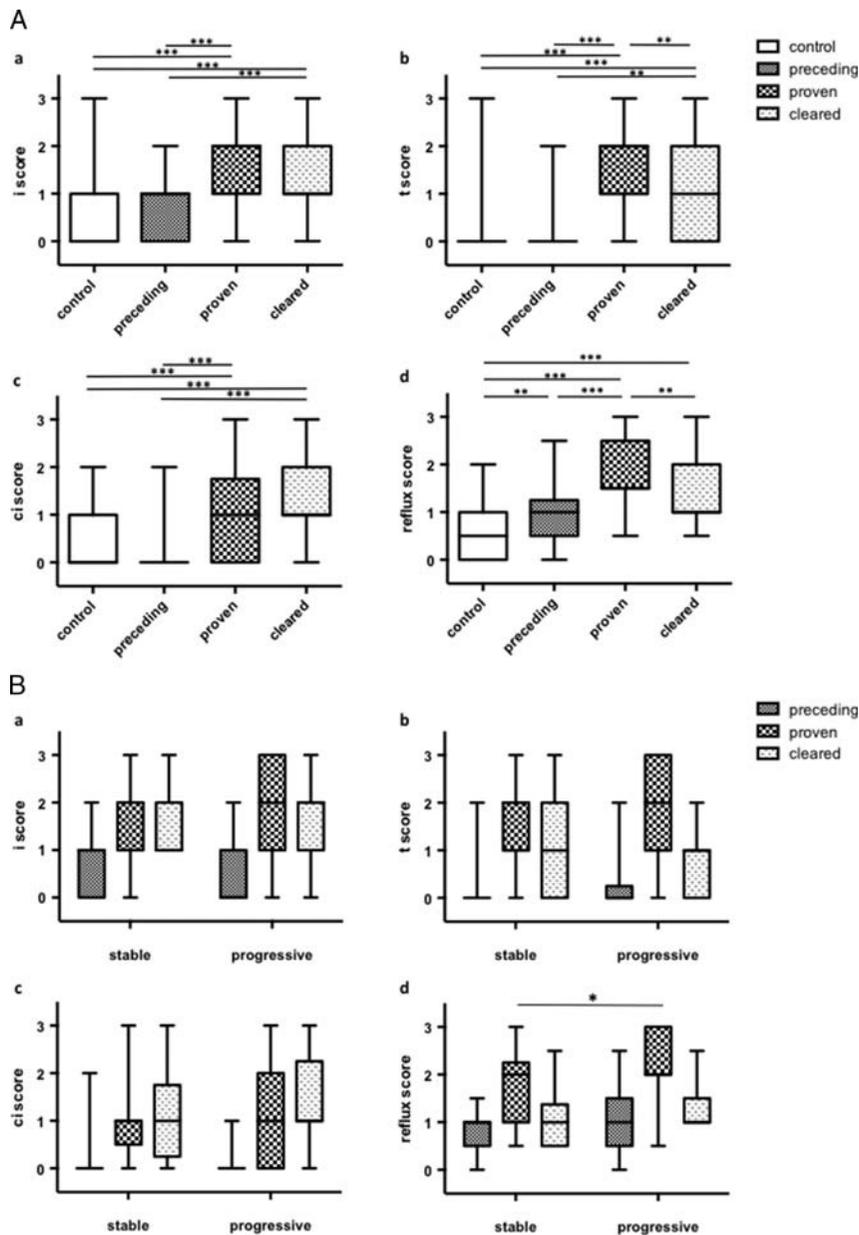
or ci scores according to Banff classification criteria (data not shown). These results indicate that reflux scores, in addition to pvl scores and BK staging, can be used in determining BKNV prognosis.

## DISCUSSION

We believe this is the first study to evaluate the association between urinary reflux and BKNV using histopathological THP immunostaining. Compared with the control group, reflux scores according to THP immunostaining in the BKNV group were higher in the preceding phase, but not in the SV40-proven stage. Reflux scores correlated with pvl scores, which reflected allograft survival in our study corroborating the observations of previous studies. Thus, reflux scores can potentially be used as a prognostic factor in BKNV patients.

Histologically, residual interstitial inflammation and intra-epithelial lymphocyte infiltration in BKNV may persist for a prolonged period after viral clearance, mimicking the pattern observed in TCMR.<sup>12</sup> In cases with the presence of endarteritis, fibrinoid vascular necrosis, peritubular capillaritis, or glomerulitis, in addition to C4d deposition within the peritubular capillaries, the coexistence of acute rejection should be considered.<sup>1</sup> The correlation between tubular HLA-DR expression and interstitial inflammation prevents the histological distinction between BKNV and TCMR.<sup>12</sup> Plasma cell infiltration in the allograft kidney is observed in

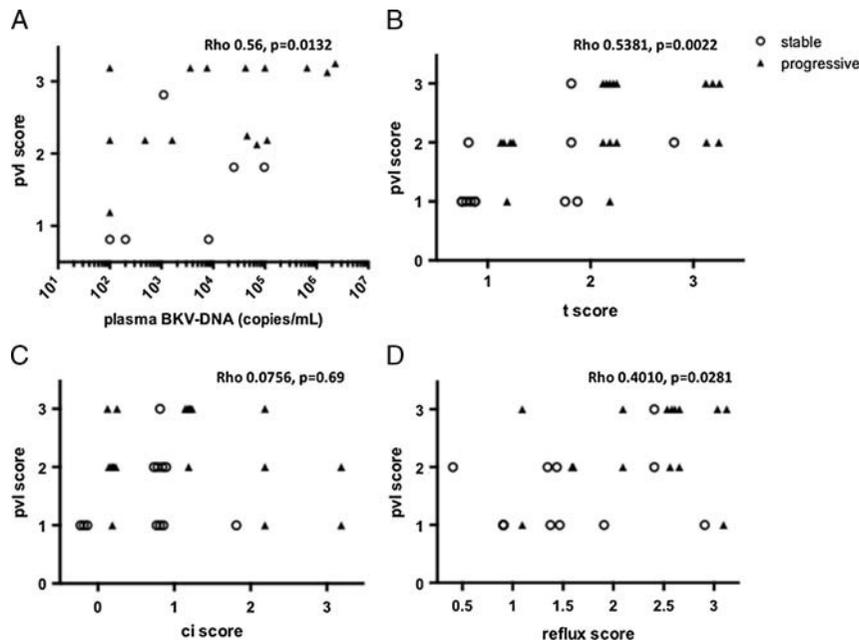
other viral infections, drug-induced tubulointerstitial nephritis, IgG4-related kidney disease, plasma cell-rich rejection,<sup>29</sup> and posttransplant lymphoproliferative disorders,<sup>30</sup> in addition to BKNV. C4d granular deposition at tubular basement membranes in BKNV,<sup>31</sup> negative peritubular capillary C4d staining, and a predominance of IgM-positive plasma cells in BKNV (IgG producing plasma cells typically predominate in rejection<sup>32</sup>) have also been reported. Although the diagnosis of TCMR remains challenging in BKNV in the context of tubulointerstitial nephritis, the distribution of BKNV differs from TCMR. Indeed, BKV is preferentially observed in the medulla or adjacent to the medullary ray area rather than the cortex. BK virus reactivation is thought to initiate in the lower urothelium (bladder or ureters) and the highly vascularized renal medulla, with later involvement of the rest of the kidney. In brief, BK virus infection spreads from the collecting duct and distal tubule to the neighborhood of the proximal tubule and glomerulus.<sup>33,34</sup> The THP staining has demonstrated utility in detecting lesions because THP expression is normally limited in the TALs of long- and short-looped nephrons, according to their proximity to vascular bundles within the inner stripe of the outer medulla. In the present study, for the evaluation of reflux nephropathy or IRR by THP staining, we established a novel histological score based on the analysis of nontransplant kidney specimens from adult and pediatric VUR cases, in addition to



**FIGURE 3.** Histological analysis of BKVN according to Banff or reflux scores. A, Histological analysis of BKVN samples compared to control samples. Banff scores (i, t, ci) were higher in SV40-proven biopsies with no significant difference observed between SV40 preceding and control biopsy samples (A-C). Reflux scores according to THP immunohistochemistry in preceding and cleared phase biopsies, in addition to SV40-proven biopsies, were higher in BKVN biopsies compared with that in control biopsies (D). B, Histological analysis of stable and progressive BKVN. A-C, No significant difference in Banff scores was observed. D, Reflux scores were higher in the progressive group compared with that in the stable group in SV40-proven biopsies. Box plot displayed the full range of variation (from min to max). \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

transplant kidney biopsy samples. In the present study, the proven BKVN group had higher i, t, and ci Banff scores and reflux scores compared with the control group. However, no correlation was observed between the Banff and reflux scores in either group except for a significant correlation between the t scores and reflux scores in the proven BKVN group (Spearman  $\rho$ , 0.3437;  $P = 0.0180$ ). The observed correlation between t scores and reflux scores was expected because t scores in BKVN are higher from the onset of disease.<sup>12</sup> We also noted the different distribution of inflammation between BKVN and TCMR cases; in fact, most cases of TCMR had no sign of urinary reflux that was measured by our method, or the area of THP obstruction in

tubuli or reflux into glomeruli was easily distinguished from the TCMR lesion; however, the i and t scores do not incorporate the disease etiology. Pathological examination of the VUR kidney (nephrectomy case) revealed the presence of striped inflammation and fibrosis predominantly in the medullary ray area (Figure 1). The term “interstitial fibrosis and tubular atrophy without any specific etiology” in the Banff classification includes various etiologies, such as CNI toxicity, chronic obstruction, and pyelonephritis<sup>35</sup>; however, the ci or ct scores do not incorporate the disease etiology and the differentiation of medullary ray fibrosis, required for appropriate management or nonspecific interstitial fibrosis and tubular atrophy, is known to be clinically challenging.



**FIGURE 4.** Histological pVI and stage in proven biopsies from stable and progressive BKVN cases. A, The pVI scores were found to significantly correlate with BK viremia. B-D, Significant correlations were found between pVI score and t scores of Banff classification or reflux scores.

Kobayashi et al<sup>17</sup> established the concept of MRI with THP staining and demonstrated it to be strongly correlated with clinical background and prognosis and to have utility in determining the appropriate management approach to CNI toxicity, UTI, or VUCG for detecting VUR. Thus, we believe our developed reflux scoring system has utility in differentiating IRR from other inflammatory states and nonspecific tubulointerstitial fibrosis.

We also observed THP reflux in Bowman capsule with crescent-like formations and infection of the parietal glomerular epithelium, as previously reported<sup>36</sup> in progressive BKVN patients, in addition to THP deposits in the tubulointerstitium which appear to derive from adjacent ruptured tubules.

The THP is extensively glycosylated, glypiated, and glycosylphosphatidylinositol anchored at the apical plasma membrane<sup>37-39</sup> and may contribute to various biological processes including receptor-mediated endocytosis,<sup>40,41</sup> mechanosensation of urinary flow, Wnt signaling, cell cycle regulation, and planar cell polarity.<sup>42</sup> The THP uses a similar mechanism to that involved in pattern recognition of pathogenic molecules, for example, transformation of immature professional antigen-presenting cells into cells with a mature phenotype by the isoreceptor of the Toll-like receptor 4.<sup>43</sup> Moreover, after exposure of the tubulointerstitium to THP, these receptors induce costimulatory molecules (CD80 and CD86) and HLA-DR molecules and contribute to proinflammatory and autoimmune responses, such as the secretion of anti-THP antibodies into the sera.<sup>43</sup> On the other hand, BKV uses N-linked glycoproteins to bind and enter host cells through caveolae-mediated endocytosis<sup>44</sup> prior to BKV capsid rearrangement and nuclear entry.<sup>45</sup> Expression of proinflammatory cytokines and chemokines is mediated by the activation of innate defense mechanisms, such as the Toll-like receptor 3 pathway<sup>46</sup>.

The presence of 3-dimensional THP-rich viral clusters, termed “Haufen,” has been recently shown to be a reliable

marker of BKVN in viremic or persistently viruric patients<sup>26</sup> and significantly correlates with histological BKVN grade.<sup>47</sup> “Haufen” are believed to be produced by cytolysis and the release of daughter virions into injured nephrons rich in THP. The THP may perform both proinflammatory and antiviral roles; however, THP may have a paradoxical effect in promoting UTI in patients with urinary catheters through the coating of catheters and facilitation of uropathogenic bacteria binding to catheters,<sup>48</sup> even if ureteral stenosis did not affect 10-year survival rates<sup>49</sup> of the patient and graft. In fact, ureteric stent placement has been shown to be significantly causative for BK viremia in a number clinical studies,<sup>13-15</sup> with speculation that the mechanical trauma associated with stent placement may injure the urothelium and allow BK viral invasion and replication through a mechanism similar to that observed in an animal model.<sup>50</sup> A viral infection dynamic model in which replication starts in the kidney then reaches the urinary tract and is followed by bidirectional viral flux into both compartments was found to be the most compatible with clinical observations.<sup>51</sup> Based on previous findings and those of the present study, we speculate that not only IRR but also THP backflow into the tubulointerstitium and Bowman capsule may subclinically promote the dispersion of BK virions into the allograft kidney.

The present study was limited by its retrospective nature, the lack of randomization, and the lack of multivariate analysis due to the small sample size. Moreover, the THP staining in the allograft kidney is not widely used and THP cast occlusion mainly in tubulus in medullary ray lesion and THP reflux into the Bowman capsule are not currently validated to be specific for IRR; it has reported mRNA expression of THP increased as part of the injury response of the nephron and THP was found in the specimen in other settings like acute kidney injury (AKI).<sup>52</sup> On the other hand, cast formation in ischemia-reperfusion model of AKI was not only unaffected but was also markedly increased in THP knockout mice.<sup>53</sup> These findings have led to suggestions that THP

may be not essential for tubular cast formation in AKI even mRNA expression of THP increases in AKI.

In the kidney transplant setting, allograft biopsies are invasive but essential for diagnosis when allograft dysfunction or abnormal urinalysis occurs. Our aim is to associate pathological findings with appropriate management approaches in cases with allograft biopsies, and we have also clarified the pathological features of reflux nephropathy not only in transplant but also in nontransplant settings and believed the concept of medullary ray injury may compensate differentiation of pathology other than rejection.

In conclusion, our preliminary data showed IRR evaluated by THP immunostaining in the allograft kidneys might have utility in the histological evaluation of BKVN after kidney transplantation, indicating that urinary reflux might be a predisposing and prognostic factor of BKVN.

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