Combined detection of urine specific gravity and BK viruria on prediction of BK polyomavirus nephropathy in kidney transplant recipients

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Abstract

Background: BK polyomavirus (BKPyV)-associated nephropathy (BKPyVAN) is an important cause of dysfunction and failure of renal transplants. This study aimed to assess the diagnostic performance of morning urine specific gravity (MUSG) in diagnosing BKPyVAN in kidney transplant recipients.

Methods: A total of 87 patients, including 27 with BKPyVAN, 22 with isolated BKPyV viruria, 18 with T cell-mediated rejection (TCMR), and 20 with stable graft function, were enrolled in the First Affiliated Hospital of Sun Yat-Sen University from March 2015 to February 2017. MUSG at biopsy and during a follow-up period of 24 months after biopsy was collected and analyzed. Receiver operating characteristic (ROC) curve analysis was used to determine the ability of MUSG to discriminate BKPyVAN.

Results: At biopsy, the MUSG of BKPyVAN group (1.008 ± 0.003) was significantly lower than that of isolated BK viruria group (1.013 ± 0.004, $P < 0.001$), TCMR group (1.011 ± 0.003, $P = 0.027$), and control group (1.014 ± 0.006, $P < 0.001$). There was no significant difference in MUSG among the isolated BK viruria group, TCMR group, and control group ($P = 0.253$). In BKPyVAN group, the timing and trend of MUSG elevate were consistent with the timing and trend of the decline of viral load in urine and plasma, reaching a statistical difference at 3 months after treatment (1.012 ± 0.003, $P < 0.001$) compared with values at diagnosis. ROC analysis indicated that the optimal cut-off value of MUSG for diagnosis of BKPyVAN was 1.009, with an area under the ROC curve (AUC) of 0.803 (95% confidence interval [CI]: 0.721–0.937). For differentiating BKPyVAN and TCMR, the optimal MUSG cut-off value was 1.010, with an AUC of 0.811 (95% CI: 0.687–0.934).

Conclusion: Combined detection of MUSG and BKPyV viruria is valuable for predicting BKPyVAN and distinguishing BKPyVAN from TCMR in renal transplant recipients.

Keywords: BK polyomavirus; Kidney transplantation; Nephropathy; Rejection; Urinalysis

Introduction

BK polyomavirus (BKPyV)-associated nephropathy (BKPyVAN) is one of the main complications after kidney transplantation, and can lead to renal dysfunction and graft failure.[1-3] Diagnosis of BKPyV infection is generally dependent on urine cytology examination, and quantitative polymerase chain reaction (PCR) to detect BKPyV-DNA in urine and plasma.[3,4] However, these techniques are not "kidney disease specific," and cannot reliably distinguish between clinically insignificant BKPyV replication and actual intra-renal disease. As such, a definitive diagnosis of BKPyVAN requires a renal biopsy.[5] However, kidney biopsy is an invasive procedure and limitations include a high risk of bleeding, it cannot be repeated frequently, and sampling error.

Morning urine specific gravity (MUSG) reliably indicates the concentration and dilution functions of the kidneys. Damage to the epithelial cells of collecting ducts and distal convoluted tubules can lead to dysfunction of urine concentration and reabsorption.[6] It has been reported that MUSG can effectively predict acute ischemic stroke and dehydration in children.[7,8] Theoretically, lytic

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BKPyV infection in the tubular epithelial cells results in dysfunction of urine concentration and reabsorption, and presents as a decline of MUSG. This decline of MUSG does not occur with BKPyV infection of the transitional epithelial cells. Therefore, the co-occurrence of BKPyV viruria and declining MUSG may indicate tubular injury caused by BKPyV infection, and suggest the occurrence of BKPyVAN.

In clinical practice, we observed that patients with BKPyVAN usually exhibit significantly lower MUSG, which rarely occurs in patients with isolated BKPyV viruria and no BKPyVAN, or in patients with T cell-mediated rejection (TCMR). It is important to discriminate patients with BKPyV viruria and BKPyVAN from those only with isolated BKPyV viruria and no BKPyVAN, because the former require immunosuppression reduction while the latter does not.[5,9] In addition, BKPyVAN and TCMR share many similar clinical and pathological characteristics, making them challenging to distinguish from each other.[10] Thus, this study was performed to determine the diagnostic performance of MUSG in the differential diagnosis of BKPyVAN, isolated BKPyV viruria, and TCMR in kidney transplant recipients.

Methods

Ethical approval

The study was approved by the Ethics Committee and the Research Board of the First Affiliated Hospital of Sun Yat-Sen University. Because of the retrospective nature, the requirement of informed consent was waived.

Study design and patients

We retrospectively reviewed the clinicopathological data of kidney transplant recipients who were diagnosed with biopsy-proven BKPyVAN between March 2015 and February 2017 in the First Affiliated Hospital of Sun Yat-Sen University. Patients with isolated BKPyV viruria but no BKPyVAN, and with biopsy-proven TCMR but negative BKPyV viruria from the time period were also included in the analysis. Patients with stable renal graft function and negative BKPyV viruria were included as a stable renal function control group.

The following inclusion criteria were used for each group. BKPyVAN group: BKPyVAN was proved by kidney biopsy, and urine BKPy viral load was $>10^3$ copies/mL. Isolated BKPyV viruria group: BKPyVAN was excluded by kidney biopsy, and urine BKPy viral load was $>10^3$ copies/mL. TCMR group: TCMR was proved by kidney biopsy according to Banff criteria, and urine BKPy viral load was $<10^3$ copies/mL. Control group: the transplanted kidney function was stable, no specific change was observed in kidney biopsy, and urine BKPy viral load was $<10^3$ copies/mL. In addition, the common exclusion criteria of the above four groups included concurrent fever, diarrhea, diabetes, diuretic drugs administration, *de novo* or recurrent glomerulonephritis, and acute/chronic pyelonephritis.

Clinical data, including MUSG, serum creatinine (Scr), and urine and plasma BKPyV-DNA load were collected from the medical record system at the time of biopsy, and at half, 1, 3, 6, 9, 12, 18, and 24 months after biopsy. Patients with other pathological injuries, and those without complete follow-up data were excluded from the analyses.

Detection of MUSG

All patients were fasted for more than 8 h before collecting urine. A total of 10 mL of morning urine was collected in a sterile tube, and tested within 2 h of collection. The MUSG was measured using a Bayer Clinitek 50 Urine Automatic Analyzer (Clinitek 50, Bayer Corporation Elkhart, IN, USA), according to the manufacturer's instructions. MUSG data were expressed in increments of 0.001, from 1.000 to 1.030. The normal range of MUSG at out center is 1.005 to 1.010.

Quantitative determination of BKPyV load

Urine and plasma BKPyV loads were quantitatively determined by quantitative PCR (q-PCR) (MJ Research, Waltham, MA, USA). Specimen collection and processing, PCR primers, TaqMan probe (targeting the BKPyV *VP1* gene), plasmid standard containing the targeted BKPyV *VP1* gene, amplification protocols, PCR precautions, and quality assurance were performed as previously described.[11] Urine and plasma BKPyV loads were presented as the BKPyV genome copies per milliliter. The limit of quantitation was 1000 copies/mL.

Diagnosis of BKPyVAN

The diagnosis of BKPyVAN was established by the presence of interstitial inflammation, tubular atrophy, interstitial fibrosis, and the extent of viral cytopathic changes in the tubular epithelial cells, and was confirmed by immunohistochemical (IHC) staining nuclear positivity with anti-SV40 large T antigen monoclonal antibody, as previously described.[12] The histological features of BKPyVAN were classified using the American Society of Transplantation schema, and BKPyVAN was classified as category A, B, and C based on the guidelines published by Hirsch *et al*.[13] Histological viral load was assessed semi-quantitatively as the percentage of tubules positive for polyomavirus using a four-tier system (<10%, 10%–25%, 25%–50%, and >50%).[14] Histological lesions were scored, and TCMR was defined using the Banff schema of renal allograft pathology.[10]

Therapy and follow-up

Patients with BKPyVAN were treated by modification of immunosuppression, including a reduction in the dosage of calcineurin inhibitor, and switching from tacrolimus to cyclosporine A or rapamycin. For patients with severe infections, the dosage of mycophenolic acid and/or calcineurin inhibitor was reduced, or the medication(s) was discontinued. Immunosuppressants were not modified in patients with isolated BKPyV viruria who were not diagnosed with BKPyVAN. Acute cellular rejection was treated with pulse methylprednisolone with or without polyclonal antibody.

All patients were regularly followed in the outpatient clinic, and Scr level, routine urinalysis, and BKPyV loads were monitored. According to the protocol at our institution, repeated biopsy was performed if the Scr level was continuously elevated, or after treatment of BKPyVAN or TCMR for 6 to 12 months.

Statistical analysis

Normally distributed continuous variables were presented as mean ± standard deviation, and non-normally distributed continuous variables as median (interquartile range). Data between groups were compared using Student's *t* test or one-way analysis of variance (if ≥3 groups) for normally distributed data, and Mann-Whitney *U* test for non-normally distributed data. Categorical data were presented as number and percentage (%), and compared by Pearson Chi-square test or Fisher exact test (if an expected value was ≤5). General linear model univariate repeated measurement data analyses were used for the comparison of the average MUSG among various groups at every follow-up time point. Receiver operating characteristic (ROC) curve analysis was used to determine the ability of MUSG to discriminate BKPyVAN. Results were reported as area under the ROC curve (AUC) and 95% confidence interval (95% CI). Correlation analysis was performed to examine the correlation between pathological score and MUSG. All analyses were two-tailed, and a value of $P < 0.050$ was considered to indicate statistical significance. All analyses were performed using IBM SPSS version 19 software (IBM Corporation, Somers, NY, USA). Sample size is calculated by PASS version 11.0 software (NCSS, USA). A sample of 18 from the positive group and 18 from the negative group achieve 91% power to detect a difference of 0.270 between the AUC under the null hypothesis of 0.500 and an AUC under the alternative hypothesis of 0.770 using a one-sided *Z*-test at a significance level of 0.050.

Results

Study cohort

A flow diagram of patient inclusion is shown in Figure 1. A total of 87 patients meeting study criteria were included in the analysis: 27 with BKPyV viruria and BKPyVAN, 22 with isolated BKPyV viruria but no BKPyVAN, 18 with TCMR, and 20 with stable graft function (control group). The baseline demographic and transplant characteristics of the patients are shown in Table 1. In the BKPyVAN group there were four cases of stage A BKPyVAN and 23 cases of stage B BKPyVAN. In the TCMR group, there were nine cases of borderline rejection, three cases of Banff IA TCMR, five cases of Banff IB TCMR, and one case of Banff IIB TCMR. There were no significant differences in age, sex, and immunosuppressive maintenance regimens between the groups (all, $P > 0.050$). There was a significant difference in baseline Scr among the four groups ($F = 26.789$, $P < 0.001$). Specifically, baseline Scr in BKPyVAN group (158.1 ± 45.0 μmol/L) was significantly higher than in the isolated BKPyV viruria group (100.3 ± 26.0 μmol/L, $P < 0.001$) and control group (94.0 ± 3.0 μmol/L, $P < 0.001$), but was similar to the level in the TCMR group (187.8 ± 58.0 μmol/L, $P = 0.053$). Baseline Scr in the isolated BKPyV viruria group was significantly lower than in the TCMR group ($P < 0.001$), but was similar to that of the control group ($P = 0.580$). Baseline Scr in the TCMR group was significantly higher than in the control group ($P < 0.001$). Urine BKPyV-DNA load ($Z = -5.427$, $P < 0.001$) and plasma BKPyV-DNA load ($Z = -4.536$, $P < 0.001$) in the BKPyVAN group were both higher than in the isolated BKPyV viruria group.

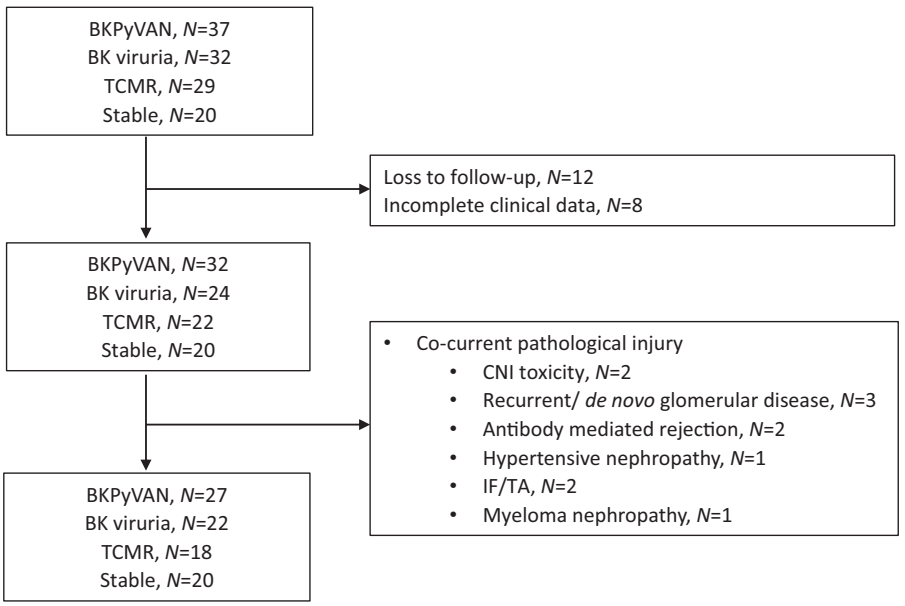

Figure 1: Flow diagram of patient inclusion. BKPyV: BK polyomavirus; BKPyVAN: BK polyomavirus-associated nephropathy; CNI: Calcineurin inhibitor; IF/TA: Interstitial fibrosis/tubular atrophy; TCMR: T cell-mediated rejection.

Table 1: Baseline demographic and transplant characteristics of the kidney transplant recipients in the four groups.

Characteristics	BKPyVAN ($n=27$)	BK viruria ($n=22$)	TCMR ($n=18$)	Control ($n=20$)	F, Z, or χ^2	P
Age (years)	38.0 ± 8.8	33.0 ± 12.0	35.3 ± 12.3	38.8 ± 11.8	1.150*	0.300
Gender, female/male	8/19	12/10	7/11	11/9	4.432†	0.218
Living/non-living donor	7/20	4/18	3/15	9/11	2.614†	0.455
Immunosuppressive regimen					4.755†	0.191
Tacrolimus + mycophenolic acid + steroid	27 (100)	20 (91)	18 (100)	19 (95)		
Cyclosporine + mycophenolic acid + steroid	0	2 (9)	0	1 (5)		
Scr at diagnosis (μmol/L)	158.1 ± 45.3	100.3 ± 26.2	187.8 ± 58.3	93.5 ± 2.7	26.789*	<0.001
Urine BKPyV-DNA (copies/mL)	2.9×10^9 (8.2×10^8, 9.4×10^9)	2.2×10^5 (1.1×10^4, 1.3×10^7)	–	–	–5.212‡	<0.001
Plasma BKPyV-DNA (copies/mL)	1.3×10^3 (1.9×10^2, 7.1×10^4)	0 (0, 0)	–	–	–4.392‡	<0.001

Values were shown as mean ± SD, *n*, *n* (%), or median (IQR). *F values; †χ^2 values; ‡Z values. BKPyVAN: BK polyomavirus-associated nephropathy; TCMR: T cell-mediated rejection; Scr: Serum creatinine; BKPyV: BK polyomavirus; SD: Standard deviation; IQR: Interquartile range.

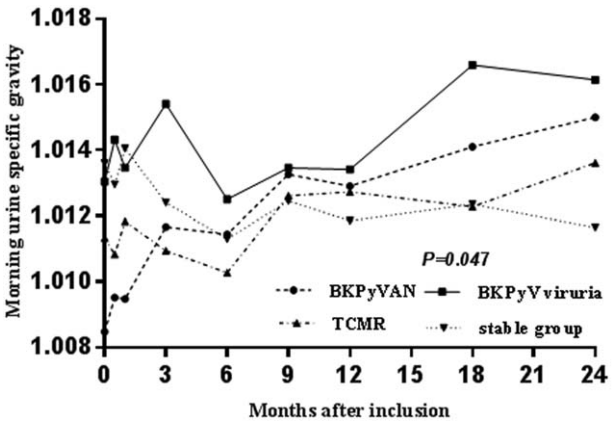

Figure 2: Morning urine specific gravity values of the four groups at each time point during the follow-up period ($P=0.047$). BKPyVAN: BK polyomavirus-associated nephropathy; TCMR: T cell-mediated rejection.

Comparison of MUSG

MUSG data of all groups are shown in Figure 2. Intra-group analysis of the BKPyVAN group showed that the MUSG gradually rose back to the normal range after modulating immunosuppressants ($F=6.724$, $P<0.001$). The MUSG at the time of biopsy (1.009 ± 0.003) was similar to that at 0.5 months (1.010 ± 0.005, $t=-0.503$, $P=0.620$), and at 1 month (1.010 ± 0.001, $t=-0.327$, $P=0.747$), but was significantly elevated at 3 months (1.012 ± 0.003, $t=-3.401$, $P=0.002$). The timing and trend of MUSG elevation were consistent with the timing and trend of urine and plasma viral load decline [Figure 3]. The result of Mantel-Haenszel Chi-square test showed a negative ($R=-0.874$, $P=0.002$) linear correlation ($\chi^2=6.107$, $P=0.013$) between MUSG and urine BKPyV-DNA load. Similarly, a negative ($R=-0.918$, $P<0.001$) linear correlation ($\chi^2=6.741$, $P=0.009$) between MUSG and plasma BKPyV-DNA load was found. In patients with BKPyVAN, there was no significant difference in MUSG during follow-up between patients receiving different treatment regimens ($F=2.467$, $P=0.131$). No patient in the isolated BKPyV viruria group developed BKPyVAN during the follow-up period.

At the time of biopsy, the MUSG varied significantly among the four groups ($F=7.489$, $P<0.001$). Specifically, the MUSG of the BKPyVAN group (1.008 ± 0.003) was significantly lower than that of the isolated BKPyV viruria group (1.013 ± 0.004, $P<0.001$), TCMR group (1.011 ± 0.003, $P=0.027$), and control group (1.014 ± 0.006, $P<0.001$) [Figure 4]. At half, 1, and 3 months the MUSG of the BKPyVAN group was significantly lower than that of the isolated BKPyV viruria group, TCMR group, and control group (all, $P<0.050$). However, the MUSG was similar between the four groups at 6 months, and at all subsequent time points (all, $P>0.050$). General linear model univariate repeated measurement data analysis showed that MUSG varied among the four groups ($F=7.262$, $P<0.001$) at 0, 0.5 month, 1 month, and 3 months. Repeated measurement of Mauchly spherical test showed that Mauchly W was 0.953 ($P=0.553$). The results of inter-class correlation coefficient (ICC) showed that ICC = 0.398 (95% CI: 0.096–0.748) in BKPyVAN group, ICC = 0.754 (95% CI: 0.529–0.887) in isolated BKPyV viruria group, ICC = 0.602 (95% CI: 0.185–0.835) in TCMR group, and ICC = 0.892 (95% CI: 0.787–0.953) in control group. There was no significant difference in MUSG among the isolated BKPyV viruria group, TCMR group, and control group at any time point ($F=2.189$, $P=0.253$).

Diagnostic performance of MUSG

The ROC analysis of MUSG for discriminating BKPyVAN and non-BKPyVAN (including isolated BKPyV viruria, TCMR, and stable renal function) showed an AUC of 0.803 (95% CI: 0.721–0.937) [Figure 5A]. Using an optimal diagnostic threshold value of 1.009, the sensitivity was 0.670 (95% CI: 0.460–0.840) and the specificity was 0.800 (95% CI: 0.680–0.890).

The ROC curve of MUSG for discriminating BKPyVAN and isolated BKPyV viruria showed an AUC of 0.852

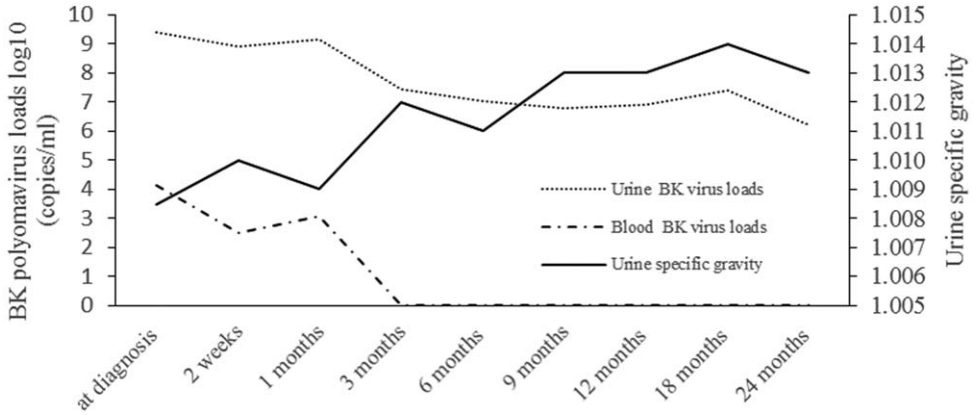

Figure 3: In patients with BKPyVAN, the timing and trend of morning urine specific gravity elevate were consistent with the timing and trend of BKPyV-DNA urine and plasma load decline. BKPyVAN: BK polyomavirus (BKPyV)-associated nephropathy.

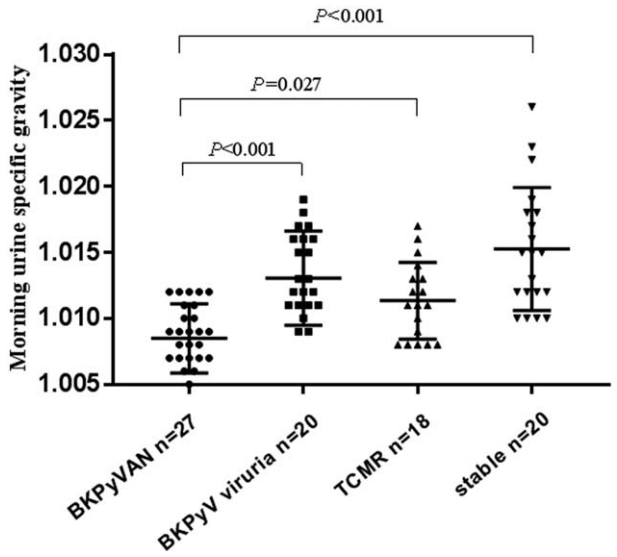

Figure 4: Morning urine specific gravity in the BKPyVAN group was significantly lower than that in the isolated BKPyV viruria group ($P < 0.001$), TCMR group ($P = 0.027$), and control group (stable graft function) ($P < 0.001$). BKPyVAN: BK polyomavirus (BKPyV)-associated nephropathy; TCMR: T cell-mediated rejection.

(95% CI: 0.726–0.940) [Figure 5B]. Using an optimal diagnostic threshold value of 1.009, the sensitivity was 0.700 (95% CI: 0.500–0.860) and the specificity was 0.860 (95% CI: 0.650–0.970).

The ROC curve of MUSG for discriminating BKPyVAN and TCMR showed an AUC of 0.811 (95% CI: 0.687–0.934) [Figure 5C]. Using an optimal diagnostic threshold value of 1.010, the sensitivity was 0.740 (95% CI: 0.540–0.890) and the specificity was 0.610 (95% CI: 0.360–0.830).

Relationship between MUSG and pathological injury in BKPyVAN

The MUSG and pathological scores at first biopsy of the BKPyVAN group were used for correlation analysis. There was no clear correlation between MUSG and tubulitis ($r = -0.161$, $P = 0.192$), interstitial inflammation ($r = -0.191$, $P = 0.122$), tubular atrophy ($r = 0.072$, $P = 0.563$), interstitial fibrosis ($r = 0.186$, $P = 0.133$), or cytopathologic change ($r = 0.062$, $P = 0.757$). There was no significant difference in MUSG among stage A, stage B1, stage B2, and stage B3 ($F = 0.582$, $P = 0.580$).

Repeated biopsies were performed in 19 patients with BKPyVAN (70.4%) 6 to 12 months after treatment. The scores of cytopathologic change at the last biopsy was significantly lower than that at first biopsy (0.579 ± 0.507 *vs.* 1.684 ± 0.820, $t = -4.996$, $P < 0.001$). Similarly, urine and plasma BKPyV-DNA loads at last biopsy were significantly lower than that at the first biopsy (urine 7.9×10^6 [2.3×10^5, 6.3×10^7] copies/mL *vs.* 2.9×10^9 [8.2×10^8, 6.9×10^9] copies/mL, $Z = -4.598$, $P < 0.001$; plasma 1.3×10^4 [2.5×10^3, 2.2×10^4] copies/mL *vs.* 0 [0, 0] copies/mL, $Z = -4.942$, $P < 0.001$). In addition, the MUSG at last biopsy was significantly higher than that at the first biopsy (1.013 ± 0.003 *vs.* 1.009 ± 0.003, $t = 3.968$, $P < 0.001$).

Discussion

Although urine analysis is a routine test during follow-up after renal transplantation, the relation between MUSG and complications has rarely been studied. In this study, we compared MUSG among kidney transplant recipients with BKPyVAN, isolated BKPyV viruria, TCMR, and stable allograft function. The results showed that MUSG significantly declined only in patients with BKPyVAN, and the value was significantly lower than that in the other three groups. Using a diagnostic threshold value of 1.009, MUSG can be used as an auxiliary indicator for predicting BKPyVAN in kidney transplant recipients with BKPyV viruria. Furthermore, using a diagnostic threshold value of 1.007, MUSG can be used to accurately distinguish BKPyVAN from TCMR.

BKPyV viruria occurs in 30.6% to 36.9% of kidney transplant recipients.[15-17] In kidney transplant recipients, BKPyV is a well-known tubulopathic virus that mainly reactivates and replicates in transitional epithelial cells and tubular epithelial cells, and replication in both is characterized by BK viruria.[18] Current tests, including

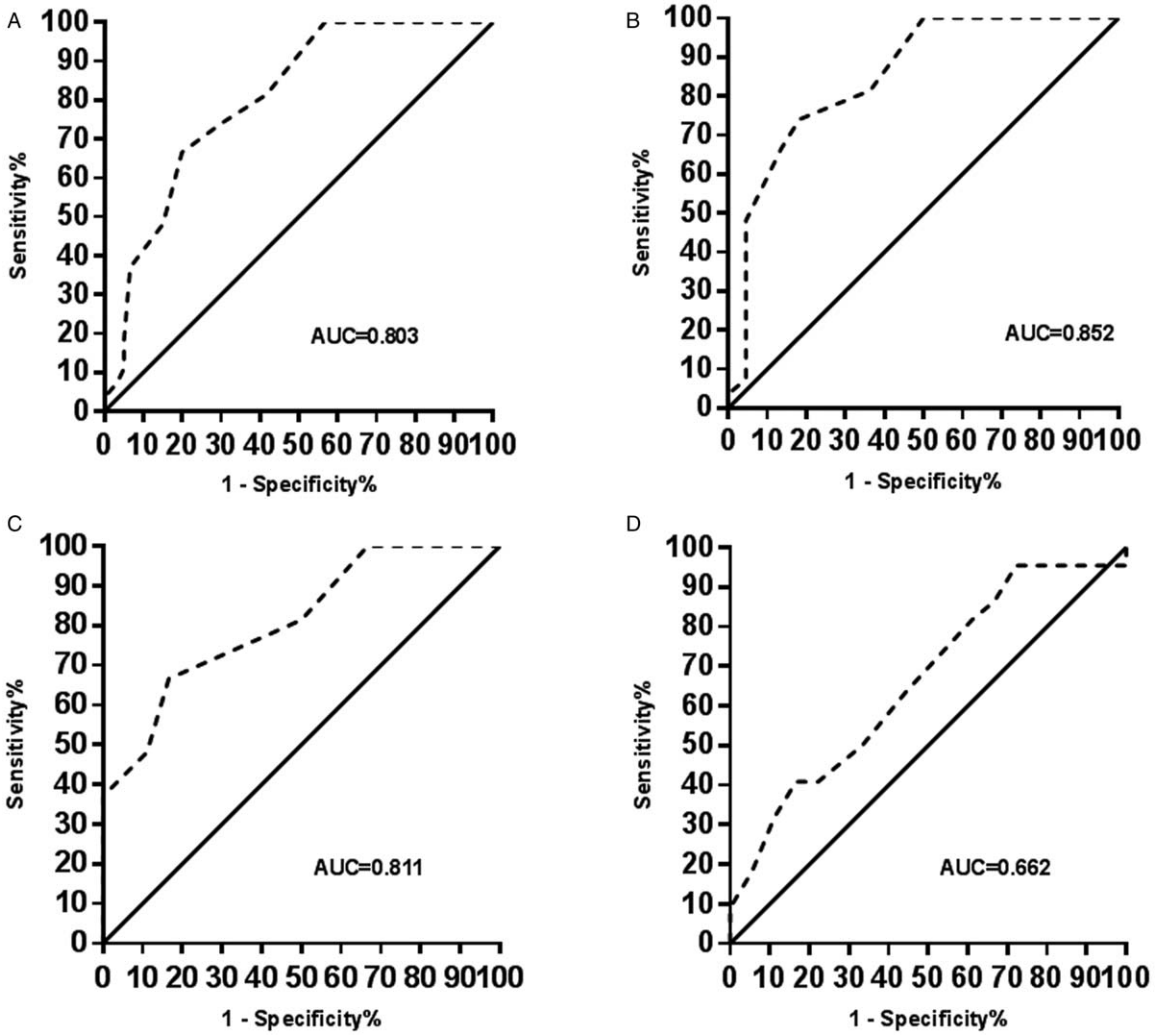

Figure 5: Receiver operating characteristic (ROC) curves of morning urine specific gravity for discriminating BKPyVAN and non-BKPyVAN (A), BKPyVAN and isolated BKPyV viruria (B), BKPyVAN and TCMR (C), and isolated BKPyV viruria and control group (D). BKPyVAN: BK polyomavirus (BKPyV)-associated nephropathy; TCMR: T cell-mediated rejection.

examination for urinary decoy cells and qPCR, can only determine whether BKPyV replicates in the urothelium; they cannot discriminate BKPyV infection in ureteral epithelial cells from BKPyV infection in tubular epithelial cells. However, only viral replication in tubular epithelial cells is destructive resulting in tubular injury, intra-graft inflammation, and development of BKPyVAN. Consequently, urine concentration is impaired which results in a decline of MUSG. Our results showed that MUSG declined significantly only in patients with BKPyVAN, and not in those with isolated BKPyV viruria but no BKPyVAN. Using a diagnostic threshold value of 1.009, the positive predictive value of MUSG for predicting BKPyVAN was 86.0%, which is higher than that of urine qPCR (40.0%) and plasma qPCR (60.0%).

Although kidney biopsy is the "gold standard" for the diagnosis of BKPyVAN, around 36.0% of cases are misdiagnosed due to sampling error.[19] The distributions of cytopathological changes, tubulointerstitial inflammation, and anti-SV40 T antigen IHC staining are localized, especially at the early stage of BKPyVAN. In this study, four patients with stage A BKPyVAN showed significant decline of MUSG, similar to that of patients with stage B BKPyVAN, indicating that MUSG decreases significantly early in BKPyVAN. Therefore, a decline of MUSG suggests BKPyV-associated tubular dysfunction when BKPyV viruria is positive. Correlation analysis showed there were no significant correlations between MUSG and indicators of pathological injury. We speculate that MUSG mainly reflect the ability of the kidney to concentrate urine, rather than pathological morphological changes.

In this study, only patients in BKPyVAN group and TCMR group required treatment. No drug for anti-rejection has a significant effect on the MUSG. In addition, all therapeutic regimens for BKPyVAN actually restore the reabsorption function of the transplant kidney by clearing plasma/urine BKPyV. Therefore, the therapeutic regimens in this study did not have a confounding effect on MUSG. Reducing the intensity of immunosuppression promotes restoration of anti-viral immunity.[5] With effective regulation, both plasma and urine BKPyV-DNA loads usually decline significantly. In our study, we found that the timing and

trend of MUSG elevate in BKPyVAN patients were consistent with the timing and trend of urine and plasma BKPyV load decline. We believe that MUSG can be used as a dynamic, real-time indicator to monitor tubular injury caused by BKPyV infection and repair of tubular function.

It is important to differentiate BKPyVAN from TCMR, because BKPyVAN is treated with a reduction of immunosuppressive drug dosages, while TCMR is treated with an increase.[5,9] However, the two conditions have overlapping histological and clinical characteristics and can be difficult to distinguish, especially when there are borderline pathological change and Banff I TCMR without endarteritis.[20] Although BKPyVAN and TCMR are both characterized by tubular epithelial injury and tubule-interstitial inflammation, BKPyV usually infects the collecting tubules and distal convoluted tubules in medulla area, while TCMR mainly involves the tubules in cortical area. Therefore, BKPyVAN causes a decline in MUSG and TCMR does not affect MUSG. The results of this study showed that a decline of MUSG was an exclusive characteristic of BKPyVAN, and the AUC of MUSG for distinguishing BKPyVAN and TCMR was 0.811.

Although proteomics[21] and genomics[22] studies have been reported to facilitate the early diagnosis of BKPyV infection, these methods have not been widely clinically validated. Moreover, the detection cost is high. In contrast, MUSG is a widely used test item in clinical practice and its cost is low. The results of this study showed that the significant reduction in MUSG has a higher diagnostic value for identification of BKPyVAN. Meanwhile, by dynamically detecting the MUSG, it can effectively reflect the change trend of the urine-concentrating function of the transplant kidney, thereby reflecting the renal tubular damage and repair process caused by BKPyV infection.

There are some factors that may influence MUSG, including: bleeding, diarrhea, fever, restricted/increased fluid intake, acute/chronic glomerulonephritis, administration of diuretic drugs, proteinuria, glycosuria, and so on.[23] However, the subjects of this study did not incorporate the above situation. In addition, all patients were negative for urine glucose at the time of enrollment. There was no significant difference in 24-h urine protein quantitation between the groups, and there was no correlation between 24-h urine protein quantitation and MUSG in this study. Therefore, we believe that there is no confounding factor that has a significant effect on MUSG in this study. Of course, in actual clinical practice, these influencing factors need to be considered.

There are some limitations in this study. This was a retrospective single-center study with a relatively small sample size. Additionally, the study only assessed kidney transplant recipients who received a biopsy. It was necessary to maximize the prevalence of BKPyVAN and TCMR in the study cohort. Further validation of the diagnostic value of MUSG for predicting BKPyVAN and for monitoring after treatment is warranted.

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Conflicts of interest

None.

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