ORIGINAL ARTICLE

Variability in assessing for BK viremia: whole blood is not reliable and plasma is not above reproach – a retrospective analysis

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SUMMARY

Polyomavirus nephropathy (PVN) is a major complication of kidney transplantation. Most reports describe polyomavirus viremia either precedes or is detectable at the time of diagnosis of PVN. This association is the basis of current screening recommendations. We retrospectively reviewed the PCR results of blood and urine samples from 29 kidney transplant recipients with biopsy-proven PVN. Biopsies were performed for a rise in serum creatinine or persistent high-level BK viruria. All biopsies showed polyoma virus large T-antigen expression in tubular epithelium using immunohistochemistry. All had viruria preceding or at the time of biopsy (range, 5.2×10^4 to $>25 \times 10^6$ BKV DNA copies/ml). Twenty (69%) had viremia ranging from 2.5×10^3 to 4.3×10^6 copies/ml at the time of the biopsy. Via blood BK PCR assay, nine (31%) had no BK viremia detected either preceding or at the time of the biopsy. In five recipients where sufficient specimen permitted, additional plasma BK assessment revealed positive detection of viremia. A comparative analysis of assays from two centres was performed with spiked samples. BK DNA may not be detected in the blood of some kidney transplant recipients with histologically confirmed PVN. This may reflect limitation of whole blood as opposed to plasma-based BK DNA assessment.

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Key words
BK virus, kidney transplant, polyoma, viremia

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Introduction

Polyomavirus-associated nephropathy (PVN) is a major agent of kidney allograft dysfunction [1–7]. Kidney dysfunction is often a late manifestation, indicating permanent damage which may progress to allograft loss [8–10]. Early diagnosis may allow intervention to prevent irreversible dysfunction [11–19]. BK viruria precedes viremia in the development of PVN, and viremia is said to be present in cases of histologically confirmed PVN [13,20–24]. Therefore, the absence of viruria and/or plasma viremia has been thought to exclude the possibility of PVN, encompassing screening guidelines [20,25].

The gold standard for diagnosis of PVN consists of allograft biopsy with identification of viral cytopathic changes on light microscopy and immunohistochemical detection of SV40 large T antigen [1,26]. Many transplant recipients do not undergo histological evaluation for PVN without the detection of viremia [23]. However, assays for BKV DNA are not standardized and consequently there may be variation in sensitivity, specificity, and predictive value [27]. We present a systematic assessment of the association between viremia, viruria, and biopsy-confirmed PVN at a single transplant center.

Materials and methods

After Institutional Review Board approval, kidney transplant recipients with PVN occurring at the University of Chicago (UC) from November 2004 to June 2007 were retrospectively identified. Immunosuppression consisted of methylprednisolone 1000 mg administered intraoperatively, with postoperative conversion to prednisone with taper over 3 months to a dose no less than 5 g/day. All patients received induction therapy with basiliximab, daclizumab or anti-thymocyte globulin, as per the discretion of the treating physician. Maintenance immunosuppression consisted of prednisone, mycophenolate mofetil (MMF) 1 g twice daily, and tacrolimus with goal trough levels of 8–10 ng/ml for the first 6 months, and 6–8 ng/ml thereafter. Antimicrobial prophylaxis consisted of fluconazole 100 mg once a day orally for 1 month post-transplant for prophylaxis against candida, and trimethoprim-sulphamethoxazole 80–400 mg daily for at least 6 months for bacterial urinary infection and *Pneumocystis jiroveci* pneumonia prophylaxis. In the setting of sulpha allergy, trimethroprim-sulphamethoxazole was substituted with inhaled pentamidine. Patients received 3 months of valganciclovir prophylaxis with goal dose of 900 mg daily, unless neither the donor nor the recipient had serologic evidence of previous exposure to CMV, in which case they received acyclovir antiviral prophylaxis.

Serum creatinine, complete blood count, tacrolimus level, and electrolytes were identified via chart review. All patients were screened for BKV in urine and whole blood using a real-time polymerase chain reaction (PCR) assay. These measurements were taken monthly for the first 3 months, then every 3 months for the first year, and thereafter yearly. If urinary BKV DNA was detected, immunosuppression dosing changes were made by the patients transplant physician. Patients were subsequently monitored for blood and urine PCR assays every 2 weeks until BK viruria resolved. Biopsies were obtained in the setting of viremia, persistent high-level viruria defined as greater than 25×10^6 copies/ml, or a rise in serum creatinine of greater than 0.4 mg/dl from baseline. Biopsies positive for PVN were classified histologically according to Drachenberg et al.'s criteria [16]. Sections were stained using a monoclonal antibody to polyoma virus SV40-T antigen, SV40-T Ag (Ab-2) (Oncogene Research Products, Cambridge, MA, USA) employing standard immunoalkaline phosphatase methods, preceded by pressure-cook antigen retrieval for 5 min in Ventana Retrieval buffer, pH 10.0. Naphthol red was used as chromogen. Sections were counterstained using haematoxylin. In instances where PVN was identified but standard in-house UC testing did not detect viremia, and where sufficient specimen permitted, plasma and urine specimens were sent to the University of Washington (UW) for confirmatory testing [27]. A subset also had in-house confirmatory assessment for plasma BKA DNA.

If PVN was identified patients received one of the following regimens per discretion of the transplant physician: (i) substitution of leflunomide for MMF and reduction in the dose of tacrolimus to a target trough level of 4–6 ng/ml; or (ii) initiation of cidofovir and reduction or discontinuation of MMF dosage and reduction of tacrolimus dosage to a target trough level of 4–6 ng/ml.

PCR assay for detection of BKV DNA (University of Chicago)

The BKV quantitative PCR assay at UC over the study period was an institutionally developed multiplex assay that detects DNA of both BK and JC virus. It was initially validated for whole blood (EDTA) and urine, as well as for CSF (qualitative JC virus results only). DNA extraction is performed using the MagNA Pure LC (Roche Diagnostic, Indianapolis, IN, USA). An initial volume of 200 µl of patient sample is extracted and

concentrated in 50 μl of eluate, using the MagNA Pure LC Total Nucleic Acid Isolation Kit. Two different known concentrations of positive samples containing BKV target, as well as a negative control containing bacterial DNA, are processed in each sample run to verify the accuracy of extraction.

The assay is specifically adapted for PCR in glass capillaries using the LightCycler Instrument (Roche Molecular Diagnostics, Pleasanton, CA, USA). A 219-bp fragment of the BKV and a 174-bp fragment of the JCV genome are amplified with specific primers and detected with probes labelled with LightCycler Red 705 (JCV) or with LightCycler Red 640 (BKV). An additional PCR product of 278 bp is formed from the internal positive control DNA (IPC) to verify the absence of amplification inhibitors in negative samples. Primers and probes are purchased from TIB MOLBIOL (Berlin, Germany) and are composed of the following:

5BKfor	ACAgCAAAgCAggCAAgg
BK rev	ggAgTCCTggTggAgTTCC
5JCfor	CTgAggAATgCATgCAgATCTA
JC rev	ggAATCCTggTggAATACA
Anchor	TTTTgCCATgAAgAAATgTTTgCCAgTAgATgA-FL
BKV	LC 640-AAgCAACAgCAgATTCTCAACACT CAACA-PH
JCV	LC 705-AAAACACAggATCCCAACACTCTACC CC-PH
IPC F	ATgCCACgTAAgCgAAACA
IPC R	gCATAAACgAAgCAgTCgAgT
IPC SS	CACTTCCCgAATAAC-FL
IPC 705	LC 705-CggATATTTTTgATCTgACCgAAgCg-PH

The target is the gene for large T antigen. Master mix is prepared using LightCycler FastStart^{PLUS} DNA Master Hybridization Probes (Roche Molecular Diagnostics). The reaction mix is 5 μl of patient eluate added to 15 μl of master mix. The protocol includes 45 cycles of PCR.

Up to six dilutions of a stock solution of cloned target DNA were processed in each run and used to generate a standard curve to determine the absolute quantification of DNA present in positive patient samples. Quantification was reported in a range from 2.5×10^3 to 25×10^6 copies/ml of patient sample; the lower and upper limits of detection of this PCR assay for BKV was 2.5×10^3 and 25×10^6 copies/ml, respectively.

PCR assay for detection of BKV DNA (University of Washington)

BK viral load testing was performed in the Clinical Molecular Virology Laboratory within the Department of Laboratory Medicine at UW. The V3T3 assay was used for BK amplification, as previously described [27]. Purified DNA was prepared by extraction at the University of Chicago (UC) or at UW using a MagnaPure Large volume DNA kit (Roche Molecular Diagnostics) with 500 μl of starting sample and a final of 100 μl, a 5-fold concentration. PCR amplification utilized two primer sets, one in the VP region (V3a) and one in the large T region (T3a), and three probes, one in the VP region (V3a) and two in the large T region (T3 and T3a) to ensure efficient amplification of all BK genotypes [27]. The Life Technologies Fast Master Mix (Roche Molecular Diagnostics) was used, primers were at a final concentration of 400 nM, probes at 100 nM, and PCR conditions were as previously described on an ABI StepOne Plus instrument (ThermoFisher Scientific, Waltham, MA, USA). The lower limit of quantification was 125 copies/ml and the lower limit of detection 25 copies/ml.

Statistical analysis

All results are expressed as mean ± standard error of the mean. Observations between the groups were compared using the unpaired *t*-test for continuous variables and Fisher's exact test for categorical variables. A two-tailed *P* value of less than 0.05 was considered significant. Categorical variables were compared using either Pearson chi-square or Fisher's exact test. Wilcoxon rank-sum test and two-sample *t*-test were used for comparison of continuous variables.

Results

Between November 2004 and June 2007, 235 patients received a kidney transplant at University of Chicago. Twenty-nine of these patients had PVN identified. All 29 had detectable BKV DNA in urine at the time of biopsy. Nine of twenty-nine had no BKV DNA detected by PCR in blood before or at the time of biopsy diagnosis (Fig. 1). Eighteen had detectable viremia at the time of or prior to biopsy diagnosis, and two had viremia detected transiently before but not at the time of biopsy.

Demographic and clinical features

There were no significant differences in the baseline demographic and characteristics between the groups, Table 1. Index biopsies tended to be obtained later in those without detectable BKV DNA in blood (median

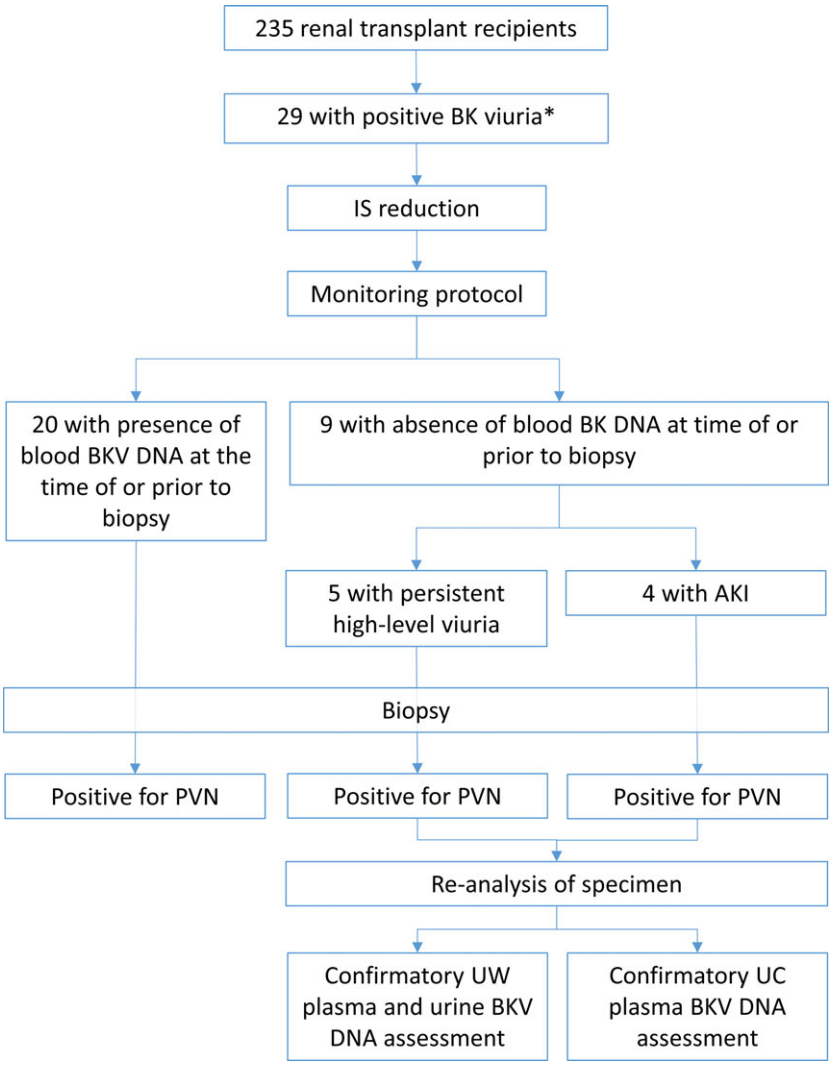

Figure 1 PVN identification and analysis. AKI, acute kidney injury, defined as rise in serum creatinine of greater than 0.4 mg/dl from baseline; IS, immunosuppression; PVN, polyomavirus nephropathy; UC, University of Chicago; UW, University of Washington. *Retrospectively identified by presence of biopsy-proven PVN. Monitoring protocol: in the setting of positive screening BK viruria, blood and urine BK PCR assays were repeated subsequently every 2 weeks until there was resolution of BK viruria. Persistent high-level viremia: as greater than 25×10^6 copies/ml.

11 vs. 3.5 months, $P = 0.08$). The frequency and the type of therapeutic agents used did not differ between the groups.

Screening of blood and urine for BKV DNA

Among the nine PVN patients with nondetectable blood BKV DNA at the time of biopsy, four had urinary BKV DNA of $>25 \times 10^6$ copies/ml at biopsy (Table 2). Two patients had transient viremia 1 month after the index biopsy with viral loads of less than 2.5×10^3 and 8.6×10^4 copies/ml, respectively. No further detectable viremia was found in subsequent testing.

In the twenty patients with detectable blood BKV DNA, one patient had viremia (9×10^3 copies/ml) detected transiently 4 months prior to the PVN diagnosis with kidney biopsy at that time showing only acute rejection. Another patient also had viremia (7×10^3 copies/ml) detected transiently 3 months prior to the diagnosis of PVN. A kidney biopsy was not performed at that time. Although no viremia was detected at the time of the index biopsy for PVN, these patients were included in this group owing to their history of previous detection of viremia.

Comparative analysis of BK aviremic whole blood specimens

Where sufficient additional specimen permitted, we performed additional analysis of whole blood aviremic for BK but with biopsy-proven PVN (Table 3). Five whole-blood specimens were available. Using the same UC assay as employed in whole-blood assessment, plasma BK assessment was performed, detecting BK viremia in two of five specimens. Using a separate plasma assay at UW, all the five had some degree of plasma BKV DNA

Table 1. Demographic data.

Variable	Group 1 n = 9	Group 2 n = 20	*P* -value
Recipient age (years; mean)	50.5	52.2	0.7
Gender (Male)	7	13	0.4
Race			
African American	4	8	
Caucasian	1	11	
Other	4	1	
Living donor	1	5	0.4
PRA > 0	1	3	1
Number HLA mismatches			
Median	5	5	0.5
25th to 75th percentile	4–6	4–5	
Thymoglobulin Induction	6	9	0.25
Basiliximab Induction	2	7	0.4
Rise in creatinine (mg/dl)	4	4	0.1
Serum creatinine at biopsy (mg/dl, median)	2.46	1.9	0.15
Time of index biopsy (months, median)	11	3.5	0.08
Maintenance immunosuppression			
Tacrolimus	9	19	1
Mycophenolate mofetil	8	19	0.5
Prednisone	9	19	1
Sirolimus	2	1	0.2
Acute rejection episodes prior to index biopsy	0	4	0.3
Delayed graft function	2	3	0.6
Acute tubular necrosis or calcineurin inhibitor toxicity	1	2	0.8

detected, albeit three at exceptionally low levels (less than 1000 copies/ml).

Assay comparison using spiked plasma samples

To assess performance characteristics, comparison was made with that of a spike plasma sample. Pooled plasma samples negative for BKV DNA were spiked with a small amount of a urine sample known to have more than 25×10^6 BKV DNA copies/ml. Serial dilutions were then prepared and assessed by the respective institution's assay (Table 4).

Indications for allograft biopsy

Although inclusion criterion for this study was the presence of PVN, this was identified retrospectively. Among persons with detectable blood BKV DNA at the time or prior to biopsy, four (20%) had concomitant rise in creatinine. Among the nine persons with detectable BK viruria but without detectable blood BKV DNA, five proceeded to biopsy owing to simultaneous rise in creatinine, and the remaining four owing to persistent high-level viruria. Patterns of PVN did not differ significantly between those with and without detectable blood BKV DNA [16].

Management after diagnosis of PVN

Immunosuppression was reduced in all patients after PVN was diagnosed. MMF was discontinued and leflunomide started in 5 patients without detectable blood BKV DNA, and 16 with detectable blood BKV DNA. One patient in the latter group was started on with cidofovir. The remaining patients in both groups were managed with reductions in immunosuppression alone.

Allograft function after diagnosis of PVN

There was no difference in serum creatinine in persons with and without detectable blood BKV DNA at biopsy with PVN (1.90 ± 0.20 vs. 2.46 ± 0.35 mg/dl; $P = 0.154$). After biopsy, among persons without detectable blood BKV DNA, two (11%) progressed to ESRD at 14 and 18 months after diagnosis of PVN. In persons with detectable blood BKV DNA, four recipients (20%) progressed to ESRD at 3, 24, 32 and 33 months after diagnosis of PVN. Overall, rates of acute rejection did not differ between those with and without detectable blood BKV DNA and PVN (20% vs. 22.2%, $P = 0.9$). One recipient with detectable blood BKV DNA suffered allograft loss in context of acute rejection, type 2A and

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Table 2. BK virus (BKV) DNA copy numbers/ml in serial specimens of blood and urines.

	Urine DNA copies/ml			Blood DNA copies/ml		
		Post biopsy			Post biopsy	
Patient	At biopsy	1 month	3 months	At biopsy	1 month	3 months
Absence of blood BKV DNA						
1	1.587×10^6	5.33×10^5	7.1×10^4	$<2.5 \times 10^3$	$<2.5 \times 10^3$	$<2.5 \times 10^3$
2	$>25 \times 10^6$	15.340×10^6	9.873×10^6	$<2.5 \times 10^3$	$<2.5 \times 10^3$	$<2.5 \times 10^3$
3	3.608×10^6	3.608×10^6	11.573×10^6	$<2.5 \times 10^3$	$<2.5 \times 10^3$	$<2.5 \times 10^3$
4	21.315×10^6	Not available	1.04×10^5	$<2.5 \times 10^3$	$<2.5 \times 10^3$	$<2.5 \times 10^3$
5	$>25 \times 10^6$	$>25 \times 10^6$	$>25 \times 10^6$	$<2.5 \times 10^3$	$<2.5 \times 10^3$	$<2.5 \times 10^3$
6	5.2×10^4	5.2×10^4	1.5×10^4	$<2.5 \times 10^3$	$<2.5 \times 10^3$	$<2.5 \times 10^3$
7	$>25 \times 10^6$	$>25 \times 10^6$	13.568×10^6	$<2.5 \times 10^3$	8.6×10^4	$<2.5 \times 10^3$
8	$>25 \times 10^6$	8.99×10^6	1.1668×10^6	$<2.5 \times 10^3$	$<2.5 \times 10^3$	$<2.5 \times 10^3$
9	3.6×10^5	4.4×10^4	Not available	$<2.5 \times 10^3$	$<2.5 \times 10^3$	$<2.5 \times 10^3$
Presence of blood BKV DNA						
1	$>25 \times 10^6$	$>25 \times 10^6$	$>25 \times 10^6$	1.1×10^4	$<2.5 \times 10^3$	$<2.5 \times 10^3$
2	$>25 \times 10^6$	$>25 \times 10^6$	$>25 \times 10^6$	1.8×10^4	5.3×10^4	$<2.5 \times 10^3$
3	$>25 \times 10^6$	5.765×10^6	18.13×10^6	1.1×10^4	$<2.5 \times 10^3$	$<2.5 \times 10^3$
4	$>25 \times 10^6$	$>25 \times 10^6$	$>25 \times 10^6$	1.1×10^5	1.8×10^4	$<2.5 \times 10^3$
5	$>25 \times 10^6$	5.195×10^6	5.41×10^5	3×10^3	$<2.5 \times 10^3$	$<2.5 \times 10^3$
6	$>25 \times 10^6$	$>25 \times 10^6$	4.77×10^5	2.52×10^5	$<2.5 \times 10^3$	$<2.5 \times 10^3$
7	$>25 \times 10^6$	6.04×10^6	6.0×10^6	7×10^3	$<2.5 \times 10^3$	$<2.5 \times 10^3$
8	$>25 \times 10^6$	$>25 \times 10^6$	$>25 \times 10^6$	4.8×10^4	$<2.5 \times 10^3$	$<2.5 \times 10^3$
9	$>25 \times 10^6$	$>25 \times 10^6$	6×10^3	1.426×10^6	$<2.5 \times 10^3$	$<2.5 \times 10^3$
10	$>25 \times 10^6$	$>25 \times 10^6$	2.09×10^2	4.36×10^6	$<2.5 \times 10^3$	$<2.5 \times 10^3$
11	$>25 \times 10^6$	$>25 \times 10^6$	$>25 \times 10^6$	3.77×10^5	4.11×10^5	$<2.5 \times 10^3$
12	$>25 \times 10^6$	$>25 \times 10^6$	Dialysis	5.24×10^5	2.93×10^5	Dialysis
13	$>25 \times 10^6$	$>25 \times 10^6$	$>25 \times 10^6$	1.124×10^6	$<2.5 \times 10^3$	$<2.5 \times 10^3$
14	$>25 \times 10^6$	$>25 \times 10^6$	$>25 \times 10^6$	$<2.5 \times 10^3$	$<2.5 \times 10^3$	$<2.5 \times 10^3$
15	$>25 \times 10^6$	$>25 \times 10^6$	19.048×10^6	1.48×10^5	2.52×10^5	$<2.5 \times 10^3$
16	$>25 \times 10^6$	11.793×10^6	4.7×10^4	$<2.5 \times 10^3$	$<2.5 \times 10^3$	$<2.5 \times 10^3$
17	$>25 \times 10^6$	Not available	39×10^6	7.01×10^5	3.08×10^5	3.35×10^4
18	$>25 \times 10^6$	$>25 \times 10^6$	6.268×10^6	2.15×10^5	1.43×10^5	$<2.5 \times 10^3$
19	9.12×10^5	Not available	2.086×10^6	$<2.5 \times 10^3$	$<2.5 \times 10^3$	$<2.5 \times 10^3$
20	8.05×10^5	2.25×10^5	2.26×10^5	$<2.5 \times 10^3$	$<2.5 \times 10^3$	$<2.5 \times 10^3$

Table 3. Comparison of assay results for BK virus (BKV) DNA copies/ml for UC and UW.

Sample #	UC whole blood	UC plasma	UW plasma
1	$<2.5 \times 10^3$	Not available	1.28×10^5
2	$<2.5 \times 10^3$	Not available	30
3	$<2.5 \times 10^3$	Not available	550
4	$<2.5 \times 10^3$	1.2×10^4	1.3×10^5
5	$<2.5 \times 10^3$	1.2×10^4	640
	UC Urine		UW Urine
1	$>25 \times 10^6$		3.0×10^{10}
2	$>25 \times 10^6$		2.6×10^7
3	$>25 \times 10^6$		2.5×10^9

UC, University of Chicago; UW, University of Washington.

PVN. Chronic kidney transplant dysfunction, defined as a persistent elevation in the serum creatinine level of 0.5 mg/dl or more from the baseline for more than 3 months, was similar between those with and without detectable blood BKV DNA and diagnosis of PVN (40% vs. 22%, $P = 0.4$).

Discussion

Current guidelines advise screening for BKV to identify persons at risk of PVN [25]. Two nucleic-acid testing based strategies are advised: screening for viremia by assessing for BK viruria, or assessing plasma for BK viremia directly. When nucleic-acid testing is not available, screening for decoy cells should be performed via urine microscopy [25]. A plasma BK level of $>10^7$ copies/ml is highly specific for PVN, to the extent that its presence is felt to be consistent with PVN and sufficient to inform changes in clinical management [1,5,25].

Table 4. BK assay comparison using spiked plasma samples.

Sample	Expected viral load (serial dilutions) DNA copies/ml	UW DNA copies/ml	UW Ct	UC DNA copies/ml	UC Ct
1	Negative	Negative	45	Negative	–
2	1×10^6	5.9×10^6	20.36	863 000	24.33
3	500 000	1.8×10^6	21.99	629 000	24.74
4	250 000	920 000	22.85	213 000	26.14
5	125 000	470 000	23.74	115 000	26.93
6	62 500	150 000	25.23	56 000	27.85
7	31 250	65 000	26.38	41 000	28.27
8	15 625	31 000	27.36	25 000	28.88
9	7813	13 000	28.53	41 000	28.26
10	3907	7400	29.28	15 000	29.58
11	Negative	Negative	–	Negative	–
12	Negative	Negative	–	Negative	–

Ct, cycle threshold; UC, University of Chicago; UW, University of Washington.

Pooled patient plasma negative for BK virus was spiked with a small amount of a urine sample previously determined to have $>25 \times 10^6$ BKV DNA copies/ml. Serial dilutions of the spiked pooled plasma sample were prepared in duplicate. One sample of each pair was assayed by UC and the other by UW.

Experts have suggested a plasma PCR threshold of $>10 \times 10^4$ copies/ml as a threshold for presumptive PVN [1,10], with a specificity of 88–98% [20,22,28]. However, it is well recognized that PVN may occur amid plasma BK viremia of $<10^4$ copies/ml. Hirsch *et al.* [22] previously described a plasma viral load of >7700 copies/ml in all patients with biopsy-proven PVN, although specific cases appeared to have even lower antecedent viremia. Singh *et al.* [29] described seven cases of PVN during which plasma BKV were detectable at low amounts ($<1 \times 10^4$ copies/ml), but no cases where BK was absent as per plasma PCR. Others too have described the imperfect reflection of PVN by way of plasma PCR [28,30–33]. A lowering of this threshold of $>10 \times 10^4$ copies/ml carries a risk of decreased specificity and consequences therein, and whereas alternate cut-offs have been proposed there has not been any direct assessment of strategies in this regard to date [1,33,34].

In our series, nine patients had PVN without blood BKV DNA detected prior to or at the time of biopsy. PVN without viremia is rarely reported [3,35,36]; previous reports have described negative plasma viremia carries a negative predictive value of >99% [1,20,22,37]. There are possible explanations for our findings which are not mutually-exclusive. The most likely one is that of a virus detection issue owing to the use of whole blood in lieu of plasma. Previous seminal reports performed assays on plasma [20,22,25,37]. Further reports suggest that whole blood is inferior to plasma for BKV DNA detection using PCR [3,27,38]. In our study a subset of specimens were re-analyzed and underwent assessment for plasma DNA (Table 3), demonstrating detection by way of plasma when negative by whole blood. Three specimens that underwent additional UW analysis had viremia identified below the UC assay's limit of detection. Via spiked plasma sample analysis both assays exhibited inaccuracy and imprecision (Table 4). Notably, values varied across clinically important thresholds, particularly at or below the spike sample expected viral load of 10^4 copies/ml.

One of the challenges regarding the generalizability of our findings is the lack of standardization for BKV DNA diagnostic assays [27,39]. There are several variables that may account for these differences, including but not limited to comparison of different sample types (urine versus urine sediment versus unextracted urine; plasma versus serum versus whole blood), the method used for DNA extraction and purification, variation in viral strains and target sequences, and the method of DNA preparation to generate standard curve [39]. There is an ongoing discussion regarding optimal thresholds and procedures [28,33,39–42]. Published cut-off values in blood and urine specimens to guide therapy should be viewed with caution in the setting of inter-institutional heterogeneity.

Intermittent viremia was described in our cohort, as has been described by others [24,43]. As opposed to intermittent urinary viruria, which may reflect intermittent shedding, the biologic plausibility for transient (as

opposed to persistent) viremia and progression to PVN is unclear. Viral kinetics and clinical significance of transient viremia are increasingly described [24] but remain an area in need for further investigation. We cannot exclude this characterization as reflective of an assay detection given our use of whole blood, as opposed being reflective of a feature of the BK virus life cycle.

It is theoretically possible that nephropathy in the setting of aviremic blood BK DNA reflects a separate causative polyomavirus (e.g. SV40, JC virus). However, the later demonstration of BKV DNA on re-assessment of plasma, when available, suggests BKV as the causative aetiology. Further, we expect JC DNA to have been detected using the UC assay given the use of specific primers in this respect. As we did not have sufficient specimen to perform such additional analysis on all specimens, this remains a limitation of our study.

Strengths of our study include the high degree of discrete patient data with quantification, and comparative confirmatory analysis across institutions. Limitations include relatively small sample size and use of blood-based DNA BKV assays as opposed to plasma-based assay. Ultimately if a standardized reference test procedure was to emerge, there would be an opportunity for comparison between screening strategies. Such analysis might include differing thresholds for intervention, as well as in comparison with existing alternate strategies (screening for decoy cells in urine, screening by way of urine BK DNA quantification) and emerging diagnostics such as quantitative urinary polyomavirus-haufen testing [29,32].

BK viruria is an effective modality for screening renal transplant recipients at risk of PVN. The absence of detectable blood BKV DNA does not exclude PVN. Plasma is the only acceptable peripheral specimen type for the assessment of viremia. As assays evolve and there are no defined reference ranges or standards across assays, comparison across institutions remains limited. Although likely less pronounced, these limitations may also be present with plasma BKV DNA assessment and may involve clinically relevant thresholds. These findings represent a potential limitation of viremia-based screening protocols, highlighting a need for international standardization of the assay.

Authorship

NA, SMM, APL, AC, RCH, BJ, PK, SM, MAJ: participated in research design. NA, IAE, SMM, APL, AC, RCH, BJ, PK, SM, JW, MAJ: participated in the writing of the paper. NA, SMM, APL, AC, RCH, PK, LC, SM, MAJ: participated in the performance of the research. NA, IAE, SMM, APL, AC, PK, LC, SM, MAJ: participated in data analysis.

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Conflicts of interest

The authors report no relevant conflicts of interest to disclose.

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