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The 2018 Banff Working Group classification of definitive polyomavirus nephropathy: A multicenter validation study in the modern era

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Polyomavirus nephropathy (PVN) remained inadequately classified until 2018 when the Banff Working Group published a new 3-tier morphologic classification scheme derived from in-depth statistical analysis of a large multinational patient cohort. Here we report a multicenter "modern-era" validation study that included 99 patients with definitive PVN transplanted post January 1, 2009 and followed the original 2018 study design. Results validate the PVN classification, that is, the 3 PVN disease classes predicted clinical presentation, allograft function, and outcome independent of therapeutic intervention. PVN class 1 compared to classes 2 and 3 was diagnosed earlier (16.9 weeks posttransplant [median], $P = .004$), and showed significantly better function at 24 months postindex biopsy (serum creatinine 1.75 mg/dl, geometric mean, vs class 2: $P = .037$, vs class 3: $P = .013$). Class 1 presented during long-term follow-up with a low graft failure rate: 5% class 1, vs 30% class 2, vs 50% class 3 ($P = .009$). Persistent PVN was associated with an increased risk for graft failure (and functional decline in class 2 at 24 months postdiagnosis; serum creatinine with persistence: 2.48 mg/dL vs 1.65 with clearance, geometric means, $P = .018$). In conclusion, we validate the 2018 Banff Working Group PVN classification that provides significant clinical information and enhances comparative data analysis.

KEYWORDS

classification systems: Banff classification, clinical research/practice, complication: infectious, infection and infectious agents - viral: BK/JC/polyoma, infectious disease, kidney disease, kidney transplantation/nephrology, pathology/histopathology, translational research/science

Abbreviations: BKPyV, polyomavirus-BK-strain; eGFR, estimated glomerular filtration rate; IHC, immunohistochemistry; PCR, polymerase chain reaction; PV, polyomavirus; PvI, polyomavirus load level; PVN, polyomavirus nephropathy; MMRM, mixed-effects model for repeated measures; S-Cr, serum creatinine; SV40-T, simian virus 40 large-T antigen.

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1 | INTRODUCTION

Renal allograft recipients, immunosuppressed with potent drugs or following desensitization, often present with polyomavirus nephropathy (PVN) as an infectious complication.[1-5] In western countries the incidence of biopsy proven so-called "definitive" PVN was estimated to be 6% with considerable variation among transplant centers.[4-6] At the University of North Carolina in Chapel Hill (UNC), we noticed an increase in the incidence of "definitive PVN" in nonsensitized ABO compatible adult renal graft recipients from 6.6% in the years 2009-2011 to 8.6% in the years 2018-2019. PVN is typically caused by intrarenal replication of the BK-virus (BKPyV) strain and has been associated with graft failure in 30%-58.3% of patients.[2,7] PVN can also affect native kidneys in severely immunocompromised patients.[8,9]

"Definitive" PVN, defined as morphologic evidence of intrarenal viral replication by light microscopy and/or immunohistochemistry (IHC), can present with different morphologic phenotypes ranging from mild and focal to florid to chronic changes.[1,10,11] Early cases may be identified only by IHC, that is, the detection of BKPyV replication based on large-T antigen expression. In order to correlate PVN phenotypes with outcome, in the past single center expert-based classification attempts were introduced that did not, however, gain broad acceptance. The goal of systematically developing a clinically significant morphologic PVN classification scheme was subsequently addressed by the Banff community. A multicenter, multinational Banff Working Group composed of nephrologists and pathologists retrospectively analyzed patients with "definitive" PVN and developed a novel 3-tier morphologic PVN classification scheme reflecting 3 clinical parameters: (1) clinical presentation at time of PVN diagnosis, (2) function during follow-up, and (3) graft loss. In contrast to previous reports, the classification was based on in-depth statistical analyses rather than expert opinion. It specifically considered renal comorbidities, such as rejection, that could have skewed morphologic definitions and distorted PVN-focused functional analyses.[7] The novel classification system was welcomed in an editorial by Dr J. Kopp as adjunct means "to provide useful prognostic information for clinical care and research applications."[12]

Because the Banff Working Group had collected and analyzed a historic patient cohort transplanted between 1996 and 2008, "modern-era" validation studies of the classification are needed.[12] Here we report such data from a multicenter study including renal allograft recipients with "definitive" PVN transplanted post January 1, 2009.

2 | MATERIALS AND METHODS

The UNC ethical review board approved this study. The study design followed the strategy described in the original Banff Working Group report.[7] Three transplant centers in the United States participated in the current validation efforts (The University of Kentucky at Lexington, The University of North Carolina at Chapel Hill, Weill-Cornell Medical Center in New York City). Each center retrospectively identified adult renal allograft recipients (≥18 years) with a biopsy proven diagnosis of "definitive" polyomavirus nephropathy: Cornell N = 30, UNC N = 54, and Kentucky N = 15 patients, total n = 99 post exclusion (see below). Some patients from Kentucky were included in a previous publication.[13] All patients were transplanted after January 1, 2009 and managed locally according to center-specific standard of care guidelines. Biopsies were obtained following local practice and preferences; they were not collected on a standardized protocol basis. In some patients with BK-viremia, biopsies were obtained from stable allografts.

Demographic, clinical, and histologic data including C4d staining and plasma BKPyV polymerase chain reaction (PCR) results, that is, BK-viremia, were provided by the participating centers. Diagnostic index biopsies in which a diagnosis of PVN was first established underwent central review (see below). Patients with evidence of acute or chronic transplant endarteritis, C4d positivity, transplant glomerulitis/glomerulopathy, acute pyelonephritis, thrombotic microangiopathy, an immune complex mediated glomerulonephritis, focal-segmental glomerulosclerosis (FSGS), diabetic nephropathy, or sepsis at time of index biopsy were excluded from the study cohort because of significant concurrent diagnoses skewing functional and outcome analyses. Note: no attempts were made to diagnose or exclude C4d negative tubulointerstial T cell–mediated Banff type 1 rejection.[14-16] Data on renal function (serum creatinine [S-Cr]) were collected before index biopsy (baseline: lowest value within 4 months before index biopsy), at time of initial diagnosis (highest available value within ± 4 days of index biopsy) and at preset intervals up to 24 months post diagnosis (1, 3, 6, 12, 24 months). BK-viremia/plasma PCR data were not collected before index biopsy but analyzed at the time of initial PVN diagnosis (highest available value within ± 14 days of index biopsy) and at the same follow-up intervals as S–Cr. S–Cr levels recorded as > 7 mg/dL or from patients with graft failure/return to dialysis were imputed with a value of 7 mg/dL (for graft failure: at time of failure and thereafter for all preset study time points). The estimated glomerular filtration rate (eGFR) was calculated using the formula of the National Kidney Foundation. Graft failure was recorded over 24 months postindex biopsy for all patients (and longer term for two thirds of the cohort). The resolution of PVN during follow-up was assessed by either a "negative" repeat biopsy or by center-determined low plasma PCR/BK-viremia reads with a cutoff of usually ≤ 250 BK copies/ml plasma. Time to PVN resolution was defined as first occurrence of a "negative" repeat biopsy or a below cutoff PCR read, whichever was reported first.

In a subgroup analysis patients with effective disease clearance (defined as having either all negative repeat biopsies or, if no follow-up biopsies were performed, BK-viremia reading below threshold within 3 months postindex biopsy) were compared to patients with PVN persistence during follow-up (defined as having all positive repeat biopsies or, in the absence of follow-up biopsies, constant PCR reads above threshold over 24 months follow-up).

The impact of severe comorbidities during 24 months follow-up, that is, Banff category 2 antibody-mediated rejection (ABMR) or

category 4 (type 2/3 T cell–mediated rejection with arteritis), pyelonephritides, glomerulonephritides, sepsis, cancer, or stroke that might have skewed data on allograft function and failure, was explored by repeating analyses on subgroups of patients post exclusion.

2.1 | Biopsy analyses

All biopsies fulfilled Banff adequacy criteria.[17] Posttransplant, all centers conducted BK-viremia testing at certain locally determined intervals to assess the developing risk for PVN. At time of original diagnostic workup allograft biopsies were analyzed by IHC with an antibody for simian virus 40 large-T antigen (SV40-T) when PVN was morphologically suspected and in all equivocal cases including in patients at increased clinical risk for PVN. A morphologic diagnosis of PVN was made based on a characteristic intra nuclear staining reaction for SV40-T antigen (epithelial cells in renal cortex or medulla) that was in 57% of cases accompanied by typical intranuclear viral inclusion bodies (Table 1). Index biopsies (initial diagnostic biopsies with PVN) were centrally reviewed at UNC (VN, HKS; hematoxylin and eosin, periodic acid Schiff, trichrome, SV40-T stains) and scored according to Banff criteria.[17,18] Assessment of the morphologic intrarenal polyomavirus load level ("pvl") was conducted as previously defined by the Banff Working Group.[7] In brief, pvl was semiquantitatively assessed based on the overall percentage of tubules in the cortex and medulla with morphologic evidence of polyomavirus replication (pvl score 1: ≤1%; pvl score 2: >1% and ≤ 10%; pvl score 3: >10% positive tubules/ducts). *A tubule* with a diagnostic IHC staining reaction for SV40-T antigen and/or a typical intranuclear viral inclusion body by light microscopy in ≥ 1 tubular epithelial cell/per tubular cross section was considered "*1 positive tubule.*" Because IHC for SV40-T antigen has a higher sensitivity due to early detection of proteins associated with viral replication, the pvl score is mainly influenced by IHC and less by the presence of intranuclear viral inclusion bodies. The overall percentage of positive tubular cross sections was estimated in the entire biopsy sample, that is, in all available cores whether affected or nonaffected/cortex and medulla. Cores were scanned at 10x magnification and the presence of positive tubules/cells was confirmed at 20x or 40x. PVN classes were defined as outlined previously (Table 2; Figure 1).[7]

2.2 | Statistical analysis

Index biopsies were used for defining PVN disease classes and for statistical associations with clinical data. Descriptive statistics are presented, with medians and interquartile ranges (IQR) for continuous variables, counts and percentages for categorical variables. Analysis of graft function over time was performed using mixed-effects model for repeated measures (MMRM) with fixed effects for study center, visit month, PVN class, and PVN class-by-visit interaction and a continuous fixed covariate for the baseline S-Cr reading.

TABLE 1 Demographics and baseline characteristics[a,b]

Age (N = 99)	**Median**	**54**
	IQR	46-64
Male (N = 99)	N (%)	64 (65)
Race (N = 99)		
White	N (%)	43 (43)
Black	N (%)	41 (41)
Latino	N (%)	4 (4)
Asian	N (%)	5 (5)
Other	N (%)	6 (6)
Donor source (N = 99)		
Deceased	N (%)	71 (72)
Living—related	N (%)	4 (4)
Living—unrelated	N (%)	24 (24)
Week of PVN index biopsy posttransplant (N = 99)	Median	24.5
	IQR	13.6-49.0
At PVN index biopsy:		
>15% increase in S-Cr over baseline (N = 99)	N (%)	65 (66)
Lowest eGFR reading (N = 99)	Median	40.81
	IQR	29.61-55.45
Plasma PCR readings ($\times 10^4$) (N = 75)	Median	2.91
(BKPyV copies/mL)	IQR	0.76-12.15
Biopsy lacking viral inclusion bodies (N = 99)	N (%)	43 (43)
Cases with at least 2 biopsy cores in index biopsy (N = 99)	N (%)	92 (93)
Diagnostic PVN changes limited to 1 core (N = 92)	N (%)	27 (29)
Diagnostic PVN changes only in medulla (N = 74)	N (%)	17 (23)
Follow-up of 24 mo post PVN index biopsy		
Allograft failure (N = 99)	N (%)	8 (8)
PVN resolution by biopsy or plasma PCR (N = 95)	N (%)	54 (57)
If resolution occurred: Time to clearance (wks) (N = 54)	Median	28.2
	IQR	16.6-61.3

Abbreviations: BkPyV, polyomavirus-BK-strain; eGFR, estimated glomerular filtration rate; PCR, polymerase chain reaction; PVN, polyomavirus nephropathy; S-Cr, serum creatinine.

[a]Medians, interquartile ranges (IQR—25th percentile to 75th percentile) are given for continuous variables, counts and percentages for categorical variables. Percentages are based on the number of subjects with data available.

[b]Sample size (N) for complete cohort is 99 subjects, with changes in sample size due to either subgroup analyses or missing data.

Because of their skewed nature, all S-Cr (and eGFR) levels were log-transformed. All statistical tests were 2 sided with $\alpha = 0.050$. (See supporting information for additional detail.)

TABLE 2 Definition of PVN disease classes

Polyomavirus nephropathy classes[a]					
Class I		Class II		Class III	
pvl	Banff ci score	pvl	Banff ci score	pvl	Banff ci score
1	0-1	1	2-3	–	–
–	–	2	0-3	–	–
–	–	3	0-1	3	2-3

Abbreviations: pvl: morphologic intrarenal polyomavirus load levels; pvl score 0: no PV replication—no PVN (no viral inclusion bodies and no SV40-T staining by IHC); pvl score 1: ≤1% positive tubules/ducts with evidence of PV replication; pvl score 2: >1 and ≤ 10% positive tubules/ducts; pvl score 3: >10% positive tubules/ducts.

Abbreviations: IHC, immunohistochemistry; pvl, polyomavirus load level; PVN, polyomavirus nephropathy; SV40-T, simian virus 40 large-T antigen.

Banff interstitial fibrosis ci-scores[17]: Ci0: interstitial fibrosis in ≤ 5% of cortical area; Ci1: interstitial fibrosis in > 5% and ≤ 25% of cortical area; Ci2: interstitial fibrosis in > 25% and ≤ 50% of cortical area; Ci3:interstitial fibrosis in > 50% of cortical area.

[a]Defined by the "Banff Working Group on Polyomavirus Nephropathy."[7]

3 | RESULTS

The study cohort consisted of 99 adult patients (median age 54 years, 43% white, 65% male, 72% grafts of deceased donor origin). An initial index biopsy diagnosis of PVN was rendered 24.5 weeks post grafting (range: 6.4-254.6 weeks; approximately 50% of patients diagnosed between 12 and 52 weeks). Per definition, diagnostic IHC staining for SV40-T antigen was detected in all cases; typical intranuclear viral inclusion bodies were noted in 57% of biopsies. In 93% of study subjects at least 2 biopsy cores were collected with diagnostic PVN changes limited to 1 core in 29% of patients and to the renal medulla in 23% (Table 1). PVN class distribution: class 1—34/99 (34%), class 2—54/99 (55%), and class 3—11/99 (11%).

Before index biopsy, 98/99 patients were on an immunosuppressive protocol containing tacrolimus; all patients received mycophenolate mofetil; 38/99 were on triple immunosuppressive therapy (tacrolimus, mycophenolate mofetil, steroids); 1/99 received sirolimus; and 54/99 were on a steroid-free protocol. Postindex biopsy immunosuppression was lowered in 84/99 patients (mainly reduction or discontinuation of cellcept or tacrolimus) and altered in 13/99, mainly by replacing cellcept with sirolimus. Immunosuppression remained

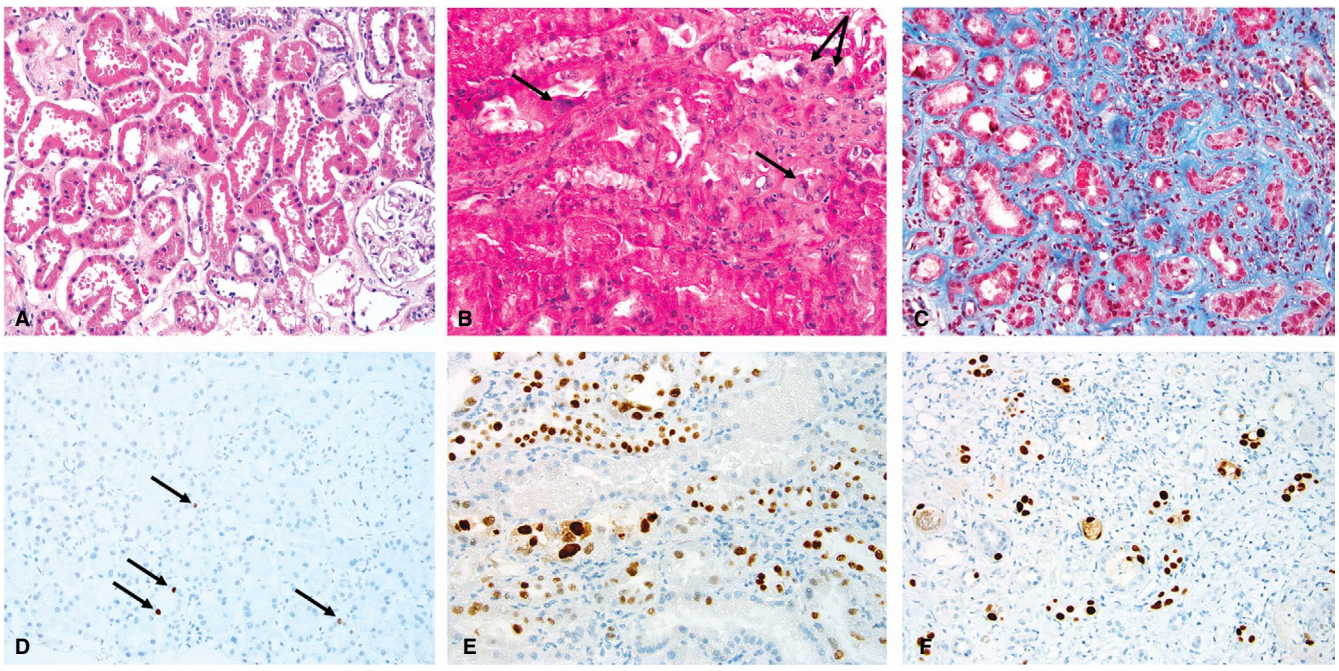

FIGURE 1 Histology of polyomavirus nephropathy (PVN) disease classes. PVN Class 1 (A/D): Renal cortex lacking significant changes including diagnostic intra nuclear viral inclusion bodies in a hematoxylin and eosin (H&E) stained section (A). Immunohistochemistry (IHC; SV40-T) shows evidence of PV replication (intranuclear staining pattern) in several tubular cross sections (arrows in D). The overall biopsy findings were scored as pvl: 1, ci: 0; 10× original magnification. PVN Class 2 (B/E): Tubules with few intra nuclear viral inclusion bodies in an H&E stained section (arrows in B). IHC (SV40-T) shows evidence of PV replication (intra nuclear staining pattern) in many tubular cross sections (E). Note: only minimal inflammation is present. The overall biopsy findings were scored as pvl: 3, ci: 1; 20× original magnification. PVN Class 3 (C/F): Renal cortex with diffuse fibrosis and tubular atrophy; diagnostic intra nuclear viral inclusion bodies are not present (C; Masson Trichrome stain). IHC (SV40-T) shows evidence of PV replication (intranuclear staining pattern) in many tubular cross sections (F). The overall biopsy findings were scored as pvl: 3, ci: 3; 10× original magnification. LS, least squares; pvl, polyomavirus load level; SV40-T, simian virus 40 large-T antigen

unchanged in 2 patients. Leflunomide, cidofovir, or IV immunoglobulins were administered in 84/99 patients, and 15 of these patients were additionally treated with fluoroquinolone. No significant differences in therapy were noted among PVN classes pre- and postindex biopsy.

3.1 | Clinical presentation at time of index biopsy

Baseline allograft function did not differ significantly between the PVN classes (class 1:1.25 mg/dL, class 2:1.38 mg/dL, class 3:1.39 mg/dL, $P = .697$; Table 3). Timing of PVN diagnosis was

TABLE 3 Clinical presentation at time of diagnostic index biopsy

		Polyomavirus nephropathy			*P* value[b]
		Class I	Class II	Class III	
Time between transplant and PVN diagnosis (wks)	Median	16.9	24.2	58.7	.004
	IQR[a]	10.6-35.1	16.3-37.0	30.4-140.0	
	N	34	54	11	
Plasma PCR readings ($\times 10^4$) at index biopsy (BKPyV copies/mL)[c]	Median	1.39	5.06	32.50	.009
	IQR[a]	0.56-3.48	1.39-12.15	1.74-48.63	
	N	26	41	8	
Baseline S-Cr (mg/dL) within 4 mo preindex biopsy[d]	Median	1.25	1.38	1.39	.697
	IQR[a]	0.98-1.73	1.10-1.70	1.05-1.89	
	N	34	54	11	
Peak S-Cr (mg/dL) at index biopsy[e]	Median	1.53	1.95	2.37	.010
	IQR[a]	1.15-1.91	1.48-2.49	1.62-2.94	
	N	34	54	11	
Change in S-Cr (mg/dL) baseline to peak	Median	0.14	0.52	0.74	<.001
	IQR[a]	0.04-0.35	0.18-1.03	0.43-1.17	
	N	34	54	11	
% Change in S-Cr baseline to peak	Median	11	36	43	.001
	IQR[a]	3-27	14-68	36-98	
	N	34	54	11	
Patients with < 15% change in S-Cr baseline to peak	N (%)	18 (53)	14 (26)	2 (18)	.007
Baseline eGFR (mL/min/1.73 m^2) within 4 mo preindex biopsy	Median	67.82	57.44	54.36	.549
	IQR[a]	50.04-75.01	43.58-72.00	33.72-72.07	
	N	34	54	11	
Lowest eGFR (mL/min/1.73 m^2) at index biopsy	Median	48.10	35.90	30.88	.004
	IQR[a]	37.49-65.41	27.48-48.69	25.63-44.34	
	N	34	54	11	
Change in eGFR (mL/min/1.73 m^2) baseline to peak	Median	−5.36	−16.17	−19.95	.004
	IQR[a]	−15.66 to −1.59	−27.23 to −7.18	−45.54 to −11.64	
	N	34	54	11	
% Change in eGFR baseline to peak	Median	−12	−31	−35	.001
	IQR[a]	−25 to −3	−46 to −15	−56 to −31	
	N	34	54	11	

Abbreviations: BkPyV, polyomavirus-BK-strain; eGFR, estimated glomerular filtration rate; PCR, polymerase chain reaction; PVN, polyomavirus nephropathy; S-Cr, serum creatinine.

[a]IQR—Interquartile range (25th percentile to 75th percentile).

[b]*P* values for the medians based on the Kruskal-Wallis test (1-way analysis of variance of the rank scores). *P* value for < 15% change in S-Cr based on the Cochran-Mantel-Haenszel Chi-Square test for a difference in the row mean scores.

[c]Plasma PCR reads/BK-viremia at time of diagnosis taken within 14 d of index biopsy and expressed as BK-copies/mL plasma.

[d]Baseline S-Cr values are lowest readings taken within 4 mo before index biopsy. Baseline eGFR readings are calculated from the baseline S-Cr readings using the formula of the National Kidney Foundation.

[e]Peak S-Cr values are highest readings taken within 4 d of index biopsy. Lowest eGFR readings are calculated from the Peak S-Cr readings using the formula of the National Kidney Foundation.

strongly correlated with PVN class: 16.9, 24.2, and 58.7 weeks for classes 1, 2, and 3, respectively ($P = .004$). At time of index biopsy acute allograft dysfunction was most pronounced in class 3 with a median S-Cr rise of 43% over baseline compared to 36% in class 2 and 11% in class 1 ($P = .001$). Similarly, class dependent functional deterioration was also reflected by changes in the glomerular filtration rates. At time of index biopsy 53% of patients in class 1, 26% in class 2, and 18% in class 3 presented with stable graft function defined as a rise in S-Cr not exceeding 15% over baseline ($P = .007$). Plasma PCR testing/BK-viremia showed highest reads in class 3 with considerable variance of test results, especially in classes 2 and 3. (Table 3).

3.2 | Allograft function over 24 months follow-up

PVN classes 1 and 2 presented with largely stable allograft function postindex biopsy with an only modest rise in S-CR over 24 months. The significant differences in S-Cr levels and eGFR noted after 12 and 24 months follow-up (24 months, class 1: S-Cr 1.75 mg/dL, eGFR: 41.19 mL/min; Class 2: S-Cr 2.16 mg/dL, eGFR: 32.30 mL/min; S-Cr $P = .037$, eGFR $P = .049$) were largely because of functional deterioration seen during the very early disease phase (Figures 2 and 3). In contrast, PVN class 3 fared worst with a progressive decline in function (24 months, class 3: S-Cr 2.66 mg/dL, eGFR 25.26 mL/min; $P = .013$ and $P = .017$, respectively, compared to class 1; Figures 2 and 3). PVN class 3 showed greater than 50% decline of eGFR over 24 months (from 54.01 mL/min at baseline before index biopsy to 25.26 mL/min). All differences were strengthened by limiting the analysis to 78 patients after excluding individuals with severe comorbidities during the follow-up time period (Figures S1 and S2). A second subgroup analysis excluded 8 patients with graft failure during 24 months follow-up that might have disproportionately influenced recorded deterioration of S-Cr levels. Postexclusion PVN class differences remained with class 1 being significantly different from classes 2 and 3 at 12 months ($P = .001$ and .005, respectively) and from class 2 at 24 months ($P = .032$). However, postexclusion of patients with graft failure, the progressive decline of renal function in class 3 was no longer detected (Figure S3).

3.3 | Graft failure postdiagnosis

In the entire study cohort 8/99 grafts failed within 24 months post PVN diagnosis and a total of 15/63 during extended follow-up. The largest percentage of graft failures were seen in PVN class 3 in both the entire patient cohort and in a subgroup excluding patients with severe comorbidities that might have negatively affected graft survival (Table 4). Graft loss was uncommon in class 1; it occurred in 1/34 patients in the setting of persistent PVN and chronic active Banff IIA rejection (also see Section 5). Differences in graft failure rates among disease classes were already apparent 24 months postindex biopsy in patients lacking severe comorbidities ($P = .030$) and became more pronounced during long-term follow-up, reaching 50%-60% in class 3 (Table 4).

3.4 | PVN resolution

Over 24 months follow-up, 54/95 patients (57%) cleared PVN either based on a negative repeat biopsy *and/or* a below threshold

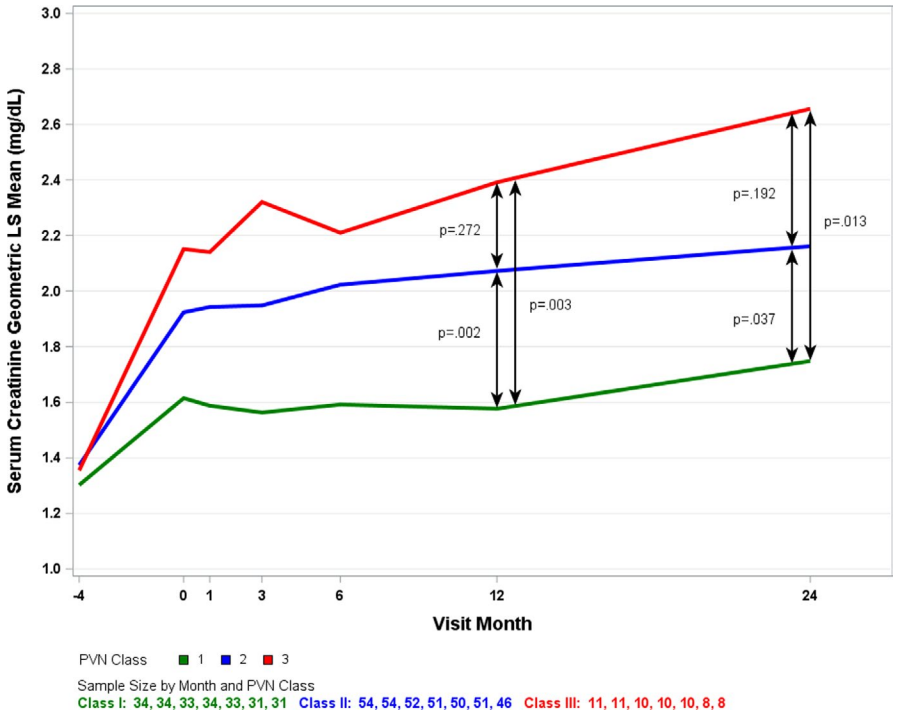

FIGURE 2 Polyomavirus nephropathy (PVN) disease classes and serum creatinine levels during follow-up. Geometric least squares mean serum creatinine (*y* axis) is plotted by visit month (*x* axis) for PVN classes 1-3 (total N = 99 patients). Significant differences in serum creatinine (S-Cr) levels are seen, highlighted here at 12 and 24 months, when comparing PVN disease classes, especially class 1 vs classes 2 and 3 (linear mixed-effects model for repeated measures [MMRM] on the log-transformed S-Cr, controlling for baseline S-Cr and study center). Baseline mean S-Cr values were determined from the exponential of the raw means of the log-transformed S-Cr readings; follow-up mean S-Cr values were calculated from the exponential of the MMRM least squares means of the log-transformed data

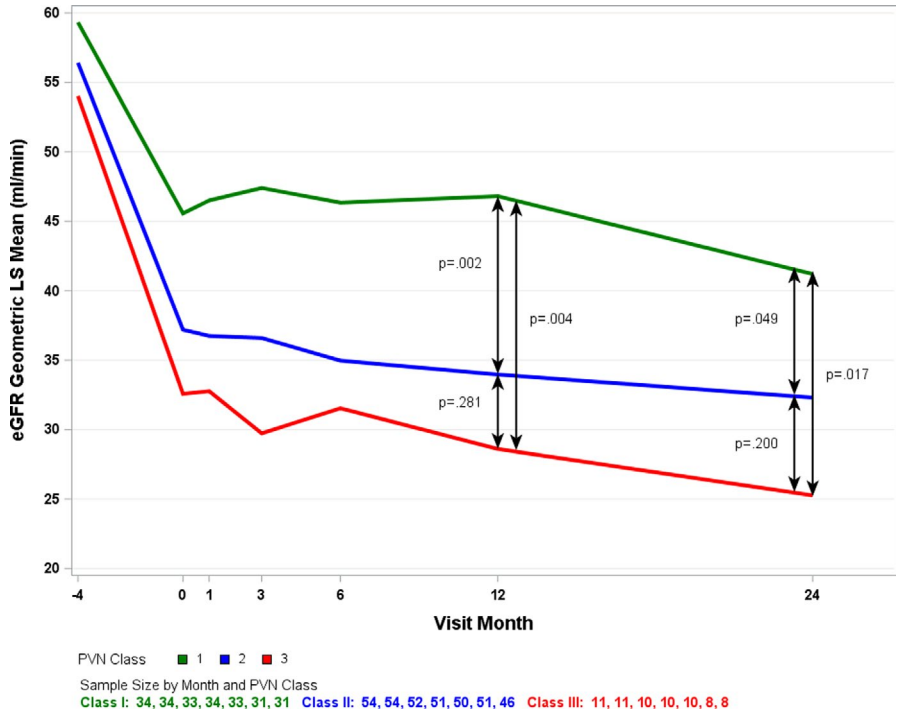

FIGURE 3 Polyomavirus nephropathy (PVN) disease classes and estimated glomerular filtration rate (eGFR) during follow-up. Geometric least squares mean estimated glomerular filtration rate (*y* axis) is plotted by visit month (*x* axis) for PVN classes 1-3 (total N = 99 patients). Significant differences in eGFR are seen, highlighted here at 12 and 24 mo, when comparing PVN disease classes, especially class 1 vs classes 2 and 3 (linear mixed-effects model for repeated measures [MMRM] on the log-transformed eGFR, controlling for baseline eGFR and study center). Baseline mean eGFR values were determined from the exponential of the raw means of the log-transformed eGFR readings; follow-up mean eGFR values were calculated from the exponential of the MMRM least squares means of the log-transformed data. LS, least squares

TABLE 4 Graft failure

PVN class		All patients: Graft failure ≤24 mo of index Bx (N = 99)	All patients: Graft failure anytime during follow-up[b] (N = 63)	Patients without other complications postindex Bx[a]: Graft failure ≤24 mo of index Bx (N = 79)	Patients without other complications postindex Bx[a]: Graft failure anytime during follow-up[b] (N = 49)
Class I	N (%)	1/34 (3)	1/20 (5)	0/28 (0)	0/16 (5)
Class II	N (%)	5/54 (9)	11/37 (30)	3/41 (7)	7/28 (25)
Class III	N (%)	2/11 (18)	3/6 (50)	2/10 (20)	3/5 (60)
	P value[c]	.098	.009	.030	.003

Abbreviations: Bx, biopsy; PVN, polyomavirus nephropathy.

[a]Excluded are patients with comorbidities during follow-up, that is, acute/chronic rejection, pyelonephritis/sepsis, recurrent/de novo renal disease, or malignancies, that might have affected graft survival

[b]Graft failure was recorded for 63 patients (n = 49 postexclusion) who were followed longer than the minimum time of 24 mo.

[c] *P* values for graft failure based on the Cochran-Mantel-Haenszel Chi-Square test for nonzero Spearman correlation using the midrank scores.

BK-viremia/PCR titer. There was a trend toward higher clearance in PVN disease class 1 that reached statistical significance in a subgroup analysis only evaluating disease resolution based on a negative repeat biopsy (class 1:76%, class 2:43%, class 3:25%, *P* = .014, Table 5). The number of patients who underwent repeat biopsy and the number of biopsies per patient did not differ significantly between PVN disease classes (*P* = .805 and 0.862, respectively, Table 6). Dependent upon the means of assessment (biopsy and/or BK-viremia), median time to PVN resolution occurred between 28.0 weeks in class 1 and 54.3 weeks in class 3 (*P* = .679, Table 5). Once PVN cleared and a repeat biopsy turned "negative," disease did not flare during further follow-up.

3.5 | The impact of disease persistence on outcome

Class 3 showed disease persistence in 3/4 patients (75%) based on apparent PVN in all postindex follow-up biopsies. In comparison,

TABLE 5 PVN resolution

		Polyomavirus nephropathy			*P* value[a]
		Class I	Class II	Class III	
PVN resolution					
By biopsy (N = 51)[b]	N (%)	13/17 (76)	13/30 (43)	1/4 (25)	.014
By biopsy or plasma PCR (N = 95)[c]	N (%)	21/31 (68)	27/53 (51)	6/11 (55)	.205
Time to PVN resolution (wks) By biopsy (N = 27)[b]	Median	38.7	36.3	53.0	.778
	IQR[d]	20.6-65.3	28.3-53.6	---	
	N	13	13	1	
By biopsy or plasma PCR (N = 54)[c]	Median	28.0	26.1	54.3	.679
	IQR[d]	18.1-70.3	13.6-39.0	20.4-67.7	
	N	21	27	6	

Abbreviations: PCR, polymerase chain reaction; PVN, polyomavirus nephropathy.

[a]*P* values for PVN Resolution based on the Cochran-Mantel-Haenszel Chi-Square test for nonzero Spearman correlation using the midrank scores. *P* values for time to PVN resolution based on the Kruskal-Wallis test (1-way analysis of variance of the rank scores). For time to PVN resolution by biopsy, the Kruskal-Wallis test is performed on PVN classes I and II only because of small sample size in class III.

[b]PVN resolution by biopsy based on all postindex biopsies through 24 mo. Patients with at least one negative biopsy for PVN during follow-up are considered to have resolved. Time to resolution is the first occurrence of a negative biopsy.

[c]PVN resolution by biopsy or plasma PCR based on at least 1 negative postindex biopsy or plasma PCR reading (below cutoff) within 24 mo of follow-up (as determined at each center), with time to resolution calculated from the PVN diagnosis to whichever measure cleared first.

[d]IQR—Interquartile range (25th percentile to 75th percentile).

TABLE 6 Postindex biopsies

		Polyomavirus nephropathy			*P* value[a]
		Class I	Class II	Class III	
Number of patients with a postindex biopsy (N = 99)	N (%)	17/34 (50)	30/54 (56)	4/11 (36)	.805
Number of follow-up biopsies per patient (N = 99)	Mean	0.85	0.74	0.82	.862
	SD	1.048	0.828	1.250	
	Range	0-3	0-3	0-3	
	N	34	54	11	
Time between index and first postindex biopsy (wks) (N = 51)[b]	Median	25	13	12	.092
	IQR[c]	15-61	7-36	3-22	
	N	17	30	4	
Patient postindex biopsy results (N = 51)[b]					
All follow-up biopsies PVN negative	N (%)	11/17 (65)	11/30 (37)	0/4 (0)	.012
All follow-up biopsies PVN positive	N (%)	4/17 (24)	17/30 (57)	3/4 (75)	.014

Abbreviations: ANOVA, analysis of variance; PVN, polyomavirus nephropathy.

[a]*P* values for frequencies based on the Cochran-Mantel-Haenszel Chi-Square test for nonzero Spearman correlation using the midrank scores. *P* values for means based on a 1-way ANOVA; *P* values for medians based on the Kruskal-Wallis test (1-way ANOVA of the rank scores).

[b]Sample size for rows 3 and 4 based on the N = 51 patients who had at least 1 postindex biopsy from row 1.

[c]IQR—Interquartile range (25th percentile to 75th percentile).

persistent PVN was found in 4/17 (24%) patients in class 1 and 17/30 (57%) in class 2 (*P* = .014). Vice versa, significantly more patients were free of PVN in all follow-up biopsies in class 1 and 2 compared to class 3 (*P* = .012; Table 6). The impact of effective PVN clearance vs persistent PVN on allograft function and survival was evaluated in a subgroup of 65 patients (Table 7). In disease class 2, effective clearance was associated with stable graft function during follow-up compared to persistent disease showing progressive functional

TABLE 7 The impact of PVN clearance or persistence on allograft failure[a]

Graft failure within 24 mo				
A. Based on postindex biopsies[b] (N = 46)		**Clearance by Bx**	**Persistent PVN by Bx**	***P* value[c]**
PVN Class I	N (%)	0/11 (0)	1/4 (25)	.024
PVN Class II	N (%)	0/11 (0)	4/17 (24)	
PVN Class III	N (%)	0/0	1/3 (33)	
B. Based on biopsies or plasma PCR[d] (N = 65)		**Clearance by Bx or PCR**	**Persistent PVN by Bx or PCR**	***P* value[c]**
PVN Class I	N (%)	0/12 (0)	1/9 (11)	.053
PVN Class II	N (%)	0/13 (0)	4/24 (17)	
PVN Class III	N (%)	0/1 (0)	1/6 (17)	

Abbreviations: Bx, biopsy; PCR, polymerase chain reaction; PVN, polyomavirus nephropathy.

[a]Table presents proportion of patients with graft failure (numerator) of those who demonstrated either PVN clearance or persistent disease (denominator).

[b]Results based on all postindex biopsies through 24 mo. Patients with only negative biopsies during follow-up are considered cleared; those with only positive biopsies are considered to have "Persistent PVN." Patients with a combination of negative and positive follow-up biopsies are excluded from this analysis.

[c]*P* values for graft failure based on the Cochran-Mantel-Haenszel Chi-Square test controlling for PVN class.

[d]Results based on biopsies if performed, otherwise on PCR readings if available. PCR readings must be negative (below cutoff) within 3 mo of index biopsy to be considered cleared. Patients with first negative PCR readings after 3 mo are considered to be neither cleared nor to have persistent PVN and are excluded from this analysis.

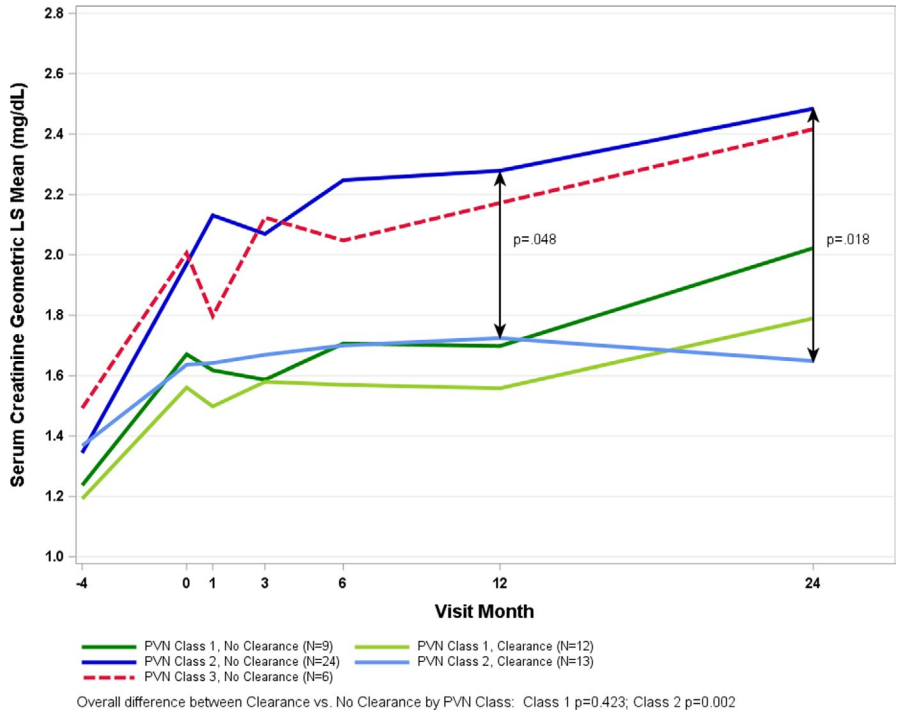

FIGURE 4 The impact of polyomavirus nephropathy (PVN) clearance or persistence on allograft function. This is a subanalysis (N = 64) examining the impact of PVN clearance on allograft function over time. Included are patients who either cleared PVN (defined by all negative repeat biopsies or, if no follow-up biopsies were performed, BK-viremia reading below threshold within 3-mo postindex biopsy) or who presented with persistent PVN during 24-month follow-up (defined as all repeat biopsies positive or, in the absence of follow-up biopsies, all reported polymerase chain reaction (PCR) reads above threshold over 24-mo follow-up). Significant differences are seen in class 2. A mixed-effects model for repeated measures [MMRM] on the log-transformed serum creatinine (S-Cr), controlling for baseline S-Cr and study center revealed overall significantly better S-Cr in patients who cleared PVN ($P = .002$). Differences to patients in class 2 with persistent disease are highlighted at 12 mo ($P = .048$) and 24 mo ($P = .018$). PVN clearance did not significantly alter allograft function during follow-up for class 1 patients. There were insufficient data for class 3 patients to make a determination. LS, least squares

deterioration (S-Cr 24 months postindex biopsy: 1.65 mg/dL with clearance vs. 2.48 mg/dL with persistence, $P = .018$; Figure 4—and also see supporting Figure S4). In class 2, the same observations were made evaluating eGFRs with significantly better filtration rates in patients with rather than without disease clearance (data not shown). Clearance also had a positive impact on function in disease class 1, although to a lesser degree. Clearance was least common in class 3 (n = 1). Very similar findings were made in a subanalysis postexclusion of patients with severe comorbidities that might have affected renal function during follow-up (Figure S4).

In the same subanalysis, the impact of PVN clearance or persistence on graft failure was studied (Table 7). All graft failures occurred in patients with persistent PVN with a strong trend toward an increased graft failure rate in class 3. Note: Case numbers in this subanalysis are relatively small.

Note: The impact of comorbidities on allograft function and outcome is shown in the supporting information section.

4 | DISCUSSION

Renal biopsies with evidence of polyomavirus nephropathy (PVN) can show various morphologies ranging from focal limited signs of viral replication to marked lytic infections to interstitial fibrosis/tubular atrophy. Although PVN phenotypes were believed to correlate with clinical parameters, PVN remained inadequately classified until 2018 when the Banff Working Group published a novel morphology-based classification system of definitive PVN.[7] The system was developed in a large multicenter and multinational cohort of patients based on comprehensive statistical analysis. In contrast to previous reports[2,3] the Banff Working Group placed emphasis on the detection of comorbidities including allograft rejection in order to also take PVN unrelated graft injury into consideration. The 3 defined PVN classes, using interstitial fibrosis and histologic polyomavirus load levels as classifiers, were predictive of clinical presentation at time of diagnosis, renal function and graft survival with favorable outcome in class 1 and an ominous disease course in class 3.

Over the last decade detailed recommendations on PVN screening and monitoring including plasma PCR testing have been introduced into patient management and PVN risk assessment[19-25]; these measures have changed clinical practice and therapeutic intervention. Thus, the objective of the current study was to test the validity of the 2018 PVN classification scheme that was primarily based on a historic patient cohort, in modern-era kidney transplant recipients.

In comparison to patients studied by the 2018 Banff Working Group, currently slightly more individuals were diagnosed with class 1 (34% vs 25% previously) and slightly less with class 2 (55% vs 63% previously) while the incidence of class 3 remained unchanged (11% vs 12% previously). The shift in incidence was presumably because of improved patient management in the modern era detecting more patients in class 1, 53% of whom presented with stable allograft function. Similar to observations published in 2018, PVN diagnosis occurred later with most pronounced acute allograft dysfunction in classes 2 and 3 compared to class 1.

Our current validation study confirmed the predictive value of the disease classes 1-3 postindex biopsy with regard to renal function in various PVN patient cohorts. At the end of follow-up 24 months postindex biopsy, allograft function was significantly better in class 1. Steepest deterioration of eGFR and S-Cr was noted in all classes during the very early disease phase followed by an attenuated decline over subsequent months in classes 1 and 2. Functional deterioration was progressive in class 3, primarily driven by those patients developing graft failure during follow-up. However, because of overall improved transplant survival in the modern era, the previously noted sharp decline in renal function in class 3 was no longer detected.

The overall graft failure rate in the entire patient cohort reported here (8%) is significantly lower than the 30% rate found in the historic patient cohort described by the Banff Working Group in 2018.[7] In both studies graft loss was highest in class 3 and lowest in class 1.

The current rate of PVN clearance, noted in 25%-76% of patients with a trend toward better and faster resolution in class 1, was similar to data reported in 2018. In a subanalysis comparing patients who effectively cleared PVN to those with persistent disease over 24 months, intriguing findings were made. Most notably, graft failure only occurred in the persistent disease cohort with highest failure rates in class 3. Persistent disease in class 2 resulted in progressive functional decline paralleling graft injury and deterioration seen in class 3. PVN class 3 had a low and slow rate of PVN resolution with overall poorest outcome. In contrast, effective disease clearance had not only a beneficial effect on graft survival but additionally also on long-term graft function in both class 2 and class 1. These data suggest, with due caution because of small case numbers, that effective PVN clearance results in greatly improved long-term outcome. The important question why some patients clear PVN more efficiently than others will have to be addressed in future studies. Certainly, the PVN class and morphologic changes are contributing factors.

All comparative clinical studies might be influenced by differences in treatment. Because specific and effective antipolyomavirus therapy is not available, patients with PVN have mainly been treated by reduction of the overall immunosuppression, and consequently outcome data seem to be less affected by differences in therapy.[21] Also in our patient cohort, stratified by class, therapy pre- and postindex biopsy was similar and therapeutic modalities cannot explain the observed PVN class differences in clinical presentation.

In addition to therapeutic intervention also comorbidities might influence the course of PVN and class presentation. Indeed, comorbidities in the historic patient cohort[7] as well as in the modern era were associated with an increased overall graft failure rate, but they did not affect the observed differences in graft loss between PVN classes. Similar to the 2018 report, in the current validation study comorbidities did not show a significant effect on allograft function beyond changes already observed in the PVN disease classes. Thus,

PVN class definitions are robust, but, nevertheless, concurrent diseases should be taken into consideration.

PCR analyses, especially on plasma samples, that is, BK-viremia tests, are of great clinical significance with established guidelines for PVN risk assessment.[25] In the context of the PVN disease classification, plasma PCR testing plays an adjunct role. One reason lies in the nature of the classification that is morphology based and in its concept similar to other histology centered classifications such as Banff, lupus, or IgA. The morphologic parameters defining PVN classes constitute a combination of various "pvl" and "ci" scores, and consequently, depending on the mix of cases especially in class 2 and 3, plasma PCR test results will show vast overlap among classes. Thus, PVN classes are imperfectly reflected by a single laboratory parameter, that is, quantitative plasma PCR test results. Furthermore, plasma PCR tests on BK-virus have limitations: (1) tests and units are not standardized and interlaboratory results can vary significantly; (2) tests target BK-virus and PVN due to JC-virus or other polyomaviruses typically remains undetected; (3) BK-viremia can originate from extrarenal tissue sites, for example, the urinary bladder or salivary glands, and consequently BK-viremia may not optimally reflect intrarenal viral disease; (4) in pediatric patients BK-viremia may reflect a primary infection with polyomavirus not associated with kidney injury or disease; (5) less than 50% of patients with viremia have "definitive" PVN including negative testing for urinary PV-Haufen[23,24]; (6) definitive PVN can be seen with low viremia of < 500 copies/mL, and vice versa no definitive PVN is reported in some patients with high titers > 10.000 copies/mL; and (7) the correlation coefficient between the level of BK-viremia/plasma PCR and the degree of intrarenal PV replication is modest ($r = .43$).[22-24] Nevertheless, plasma PCR test results provide crucial adjunct information during biopsy workup and PVN class determination.

Our current study also confirmed previous recommendations on sample adequacy and diagnostic evaluation, namely the importance of at least 2 biopsy cores including medulla for PVN workup and IHC SV40-T staining as an early screening tool for viral replication. The rational for this approach, put forward by the Banff Working Group in 2018, is supported by observations in the modern era, that is, 34% of patients presented with stable renal function at time of diagnosis and 43% of biopsies did not show intranuclear viral inclusion bodies, thus requiring IHC as an essential diagnostic element. In comparison to other adjunct techniques, such as in situ hybridization or IHC with antibodies directed against PV capsid proteins, IHC for SV40-T antigen has the advantage of detecting proteins expressed early during initial phases of PV replication that remain undetected when targeting late gene products. Because 23% of cases had PVN limited to the renal medulla and 29% of cases had PVN limited to one biopsy core, adequate material including medullary parenchyma is needed for PVN workup.

In conclusion, our modern-era validation study confirmed the validity of the 3-tier morphologic PVN classification put forward by the Banff Working Group in 2018. As a prerequisite for "validation" the current study used the same design and approach as described in detail in the 2018 report. Unique to this approach was careful consideration of comorbidities including rejection in order to best characterize the impact of PVN on graft function and outcome. In contrast to previous primarily expert-driven classification attempts[11,26,27] the basis for the current classification was in-depth statistical analysis. This PVN classification scheme can easily be used in the context of Banff lesion scoring because it is based on the Banff "ci-score" with the "pvl-score" being the only addition. Thus, PVN disease classes can readily be reported within the Banff diagnostic system.[28] As stated by the working group in 2018: "it is our contention that the proposed, readily applicable PVN classification provides prognostic information during routine biopsy workup worldwide ….(and)….should be conducted in the framework of Banff lesion scoring. It is a cornerstone to improve comparability of studies, outcome results, and therapeutic trials. A simple diagnosis of PVN in a biopsy report no longer suffices."[7]

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DISCLOSURE

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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