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ORIGINAL ARTICLE

Assessment of the Banff Working Group classification of definitive BK polyomavirus nephropathy

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SUMMARY

Polyomavirus associated nephropathy (PyVAN) continues to be a burden in renal transplantation leading to allograft insufficiency or graft failure. A presumptive diagnosis of PyVAN is made based on the presence of BK polyomavirus in patients' plasma; however, kidney biopsy remains the gold standard to establish a definitive diagnosis. The Banff Working Group on PyVAN proposed a novel classification of definitive PyVAN based on polyomavirus replication/load level and the extent of interstitial fibrosis. The aim of our study was to test the newly defined classes of PyVAN using independent cohorts of 124 kidney transplant patients with PyVAN with respect to the initial presentation and outcome, and to compare our analysis to that previously reported. Detailed analysis of our cohort revealed that the proposed classification of PyVAN did not stratify or identify patients at increased risk of allograft failure. Specifically, while class 3 was associated with the worst prognosis, there was no significant difference between the outcomes in classes 1 and 2. We also found that the timing post-transplantation and inflammation in areas of interstitial fibrosis and tubular atrophy might be additional factors contributing to an unfavorable allograft outcome in patients with PyVAN.

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BK associated polyomavirus nephropathy, renal transplantation, classification

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Introduction

The great majority of polyomavirus associated nephropathy (PyVAN) is caused by infection with BK polyomavirus and continues to be a significant burden following renal transplantation. While in the immunocompetent hosts the ubiquitous polyomaviruses usually cause clinically insignificant disease, reactivation or primary infection in immunocompromised patients leads to intrarenal infection that may result in allograft insufficiency or graft failure [1–5].

The clinical diagnosis of PyVAN is suspected (presumed) if the number of viral copies detected by serum polymerase chain reaction (PCR) is above $4_{\log}10$ BK copies/ml (or equivalent), but the definitive diagnosis of PyVAN is established based on histologic and immunohistochemical (IHC) evaluation of an allograft biopsy [5]. Pathologically, PyVAN is characterized by the presence of viral cytopathic changes accompanied by varying degrees of tubulointerstitial inflammation. Infected intrinsic renal cells, most commonly tubular epithelial cells, exhibit characteristic cytopathic effects including enlargement of the nuclei and the presence of smudgy basophilic intranuclear inclusions. The infected cells are positive for Simian virus large T antigen (SV40-T) antigen, which serves as a confirmatory stain for polyomaviruses. The inflammatory infiltrate is composed of a mixed population of mononuclear cells and often contains a significant number of plasma cells. The repertoire of the inflammatory cells, as well as the presence of tubulitis, is indistinguishable from that seen in acute T-cell mediated rejection. Therefore, the identification of infected cells via immunohistochemistry is crucial to establish the diagnosis and to implement appropriate treatment. In addition, chronic PyVAN causes accelerated interstitial fibrosis that most likely contributes to the deterioration of allograft function [6].

A few attempts to classify the lesions of PyVAN have been undertaken, but none was found to have a significant predictive value; therefore, these have not reached widespread use by the renal pathology and nephrology communities [7–10]. In 2018, the Banff Working Group on polyomavirus nephropathy proposed a novel 3-tier classification of PyVAN designed to classify the pathologic lesions associated with BK intrarenal infection and to provide prognostic information [11]. Following the comprehensive review of clinical and pathology data submitted by several centers in the United States and Europe, this new classification divided PyVAN into three classes (Table 1). The classification was based on two variables: the polyomavirus load (pvl) and the extent of interstitial fibrosis (ci score as defined by the Banff classification of allograft rejection [12]). This approach differed from previous attempts to classify this type of injury that was based on the presence of inflammation and tubular injury [9]. Both, the initial description of the classification [11] and the subsequent validation study [13] have shown significant differences in the outcomes among the newly defined groups.

Table 1. Histologic classification of biopsy-proven polyomavirus nephropathy.

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

ci, Banff interstitial fibrosis score; pvl, polyomavirus load.

Polyomavirus load – pvl 1: ≤ 1% of all tubules/ducts with viral replication, pvl 2: >1% to ≤ 10% of all tubules/ducts with viral replication, pvl 3: > 10% of all tubules/ducts with viral replication.

Banff interstitial fibrosis score – ci0: interstitial fibrosis in up to 5% of cortical area, ci1: mild, interstitial fibrosis in 6 to 25% of cortical area, ci2: moderate, interstitial fibrosis in 26 to 50% of cortical area, ci3: severe, interstitial fibrosis in more than 50% of cortical area.

The aim of our study was to test the newly defined classes of PyVAN using independent cohorts of kidney transplant patients with PyVAN with respect to the initial presentation and outcome, and to compare our analysis to that previously reported.

Materials and Methods

Study group

Four institutions participated in this study: Eastern Virginia Medical School (EVMS), Norfolk, VA; Medical University of Warsaw (MUW), Warsaw, Poland; Vanderbilt University Medical Center (VUMC), Nashville, TN; and Cedars Sinai Medical Center (CSMC), Los Angeles, CA. The study protocol has been reviewed by the appropriate ethics committee at the participating institutions. Following their approval, the study was performed in accordance with the ethical standards laid down in the 2000 Declaration of Helsinki and the Declaration of Istanbul, 2008. Since the study involved a

retrospective review of medical records, informed consent of the participating patients was waived. The inclusion criteria for our study were as follows: age ≥18 years, diagnosis of PyVAN in allograft biopsy without concurrent rejection, and available clinical data with at least 24-month follow-up after the diagnosis of PyVAN. Graft failure was defined as a return to hemodialysis or graft nephrectomy. In the clinical setting of graft failure, the serum creatinine (S-Cr) values were imputed as 7 mg/dl (for purpose of calculation), both at the time of the graft failure and at follow-up. PVN clearance was defined as BK plasma load less than 250 BK copies/ml and corresponding follow-up allograft biopsy without viral cytopathic effect and negative staining for SV40. The clinical data collected for this study included basic demographic information: age, gender, date of renal transplantation, source of transplanted kidneys, and treatment protocol. Clinical data included S-Cr level within 4 months before the index biopsy, at the time of PyVAN diagnostic (index) biopsy and at 24 months postindex biopsy, plasma BK PCR levels at the time of the biopsy and the highest (peak) level detected within 4 months from the biopsy, as well as the presence of hematuria and proteinuria. Hematuria was defined as more than 3 RBC/HPF. Protein excretion was measured as urine-protein-to creatinine ratio in spot urine. Proteinuria was defined as protein excretion of ≥300 mg/dl. Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [14].

Histologic evaluation

Histologic evaluation was performed by experienced renal pathologists at the participating medical centers without central secondary review. The histologic sections were evaluated and scored according to the Banff grading schema for rejection and the new classification of PyVAN [12,15]. In all but one center (SCMC), the pvl and ci scores were assessed by two independent pathologists, who in cases of disagreement, reviewed the case together to reach a consensus. Evidence of T-cell mediated rejection was defined as endarteritis or tubulitis and interstitial inflammation in areas remote from areas with SV40 positivity and/or viral cytopathic change. Antibody-mediated rejection (ABMR) was defined as the presence of histologic, immunohistochemical, and serologic evidence of antibody-mediated changes as defined by 2019 Banff classification for ABMR [15]. All PyVAN cases were examined for the presence of viral cytopathic effect, and the diagnosis of PyVAN was confirmed with IHC staining for SV40-T antigen. The pvl was estimated as an overall percentage of SV40-T antigen-positive tubular cross-sections in the entire biopsy sample. The pvl score was semiquantitatively assessed on a scale from 1 to 3 (Table 1) [11].

Statistical analysis

The statistical analysis of the collected data was performed by the biostatistician (IEM) at EVMS. The PyVAN classes were assigned using a formula created in Excel. The categorical variables were presented as counts and percentages. The continuous variables were expressed as medians and interquartile range (IQR).

Kruskal–Wallis test (one-way analysis of variance of the rank scores) was used to compare medians and the comparison of percentages was performed using the Cochran–Mantel–Haenszel chi-squared test for nonzero Spearman correlation. To control for a false positive rate, the Bonferroni correction method was utilized for the adjustment for multiple comparisons. Analysis of graft function over time was performed using mixed-effects model for repeated measures (MMRM) with fixed effects for visit, PyVAN class, and PyVAN class-by-visit interaction. Due to their skewed nature, S-Cr (and eGFR) levels at baseline and during follow-up were log-transformed prior to inclusion in the mixed model. This model was used to compare graft function at 12 and 24 months between the PyVAN classes, and geometric mean S-Cr (and eGFR) values were plotted against visit month by PyVAN class. Baseline S-Cr mean values (and eGFR) were determined from the exponential of the raw PVN class means of the log-transformed S-Cr (and eGFR) readings; follow-up S-Cr (and eGFR) values were calculated from the exponential of the MMRM least-squares PyVAN class means of the log-transformed data.

All statistical tests were two-sided with $\alpha = 0.050$ and were performed using SAS software, Version 9.4 (SAS Institute, Cary, NC, USA).

Results

Study group

A total of 124 renal allograft recipients, transplanted between 2004 and 2018, met the inclusion criteria (EVMS, $n = 24$; MUW, $n = 56$; VUMC, $n = 26$; and CSMC, $n = 18$). Demographic characteristics are summarized in Table 2 in the format employed in previous publications [11,16]. There were 32 (26%) females and

Table 2. Demographic and baseline characteristics.

Demographic and baseline characteristics	Measurement	Value
Age, *N* = 124	Median (IQR)	53 (41–60.25)
Male, *N* = 124	*n* (%)	92 (74)
Race, *N* = 124		
White	*n* (%)	83 (67)
Black	*n* (%)	23 (19)
Latino	*n* (%)	7 (6)
Asian	*n* (%)	9 (7)
Other	*n* (%)	2 (2)
Donor source		
Deceased	*n* (%)	102 (82)
Living-related	*n* (%)	3 (3)
Living- unrelated	*n* (%)	19 (15)
Renal bx before PyVAN index bx, *N* = 124	*n* (%)	44 (35)
Bx-proven acute allograft rejection before PyVAN, *N* = 124	*n* (%)	12 (10)
Week of PyVAN index bx post tx, *N* = 124	Median (IQR)	31 (18–62)
At PyVAN index bx		
>15% increase in S-Cr over baseline, *N* = 124	*n* (%)	103 (83)
Lowest eGFR (ml/min/1.73m^2), *N* = 124	Median (IQR)	31 (24–44.25)
Hematuria, *N* = 124	*n* (%)	23 (19)
Proteinuria, *N* = 113	*n* (%)	47 (42)
Plasma BKV PCR readings ($\times 10^4$), *N* = 118	Median (IQR)	9.8 (2.6–31.9)
PyVAN index bx lacking viral inclusion bodies, *N* = 124	*n* (%)	33 (27)
PyVAN index bx with at least two bx cores	*n* (%)	105 (85)
Diagnostic PyVAN changes limited to one core, *N* = 105	*n* (%)	22 (21)
Diagnostic PyVAN changes only in medulla, *N* = 123	*n* (%)	5 (4)
Follow-up of 24 mo post-PyVAN index bx		
Allograft failure, *N* = 124	*n* (%)	18 (14.5)
PyVAN resolution by PCR and/or bx, *N* = 119	*n* (%)	62 (52)
Time to PyVAN resolution, *N* = 119	Median (IQR)	28.4 (15–47.9)

bx, biopsy; eGFR, estimated glomerular filtration rate; IQR, interquartile range, N, sample size of the cohort with available data for the measured parameter; mo, months; n, measurement of the given parameter; PyVAN, polyomavirus nephropathy; S-cr, serum creatinine; tx, transplantation.

92 (74%) males. Most of the patients were white (67%). Most patients received the transplant from deceased donors (82%). 44 (35%) patients underwent a previous biopsy, of which 12 (27% of those biopsied; 10% of all patients) showed acute allograft rejection.

At the time of diagnosis of PyVAN, patients were receiving immunosuppressive therapy including the combination of calcineurin inhibitors (tacrolimus or cyclosporin) and mycophenolate mofetil with or without steroids. Following the detection of viremia and subsequent diagnostic biopsy, the immunosuppression was lowered in 122 (98.4%) patients. These changes included reduction/discontinuation in mycophenolate mofetil and replacement of sirolimus with cyclosporine in most patients, and in a minority of patients by alteration of the dose of steroids, with addition of rapamycin or azathioprine.

Clinical data at the time of PyVAN diagnosis

The initial indications for the allograft biopsy were persistent BK viremia and rising S-cr level. The diagnosis of PyVAN in our cohort was established after a median of 31 weeks post-transplantation (range 4–345 weeks; interquartile range, IQR 18–62 weeks; Table 2). At the time of the index biopsy, the median S-Cr was 2.1 mg/dl (compared with baseline median of 1.6 mg/dl), which represented an increase in more than 15% over the baseline in 102 (82%) of patients. The median calculated eGFR was 31 ml/min/1.73m^2 (IQR 24–44.25 ml/min/1.73m^2), and the median plasma BKV PCR reading was 9.8x10^4 (IQR 2.6–31.9). Additionally, 23 (19%) patients had hematuria, and 47 (42%) had low-level proteinuria.

PyVAN diagnostic biopsy findings

A range of 1 to 6 cores of renal parenchyma was available for examination, with 105 biopsies (85%) with at least two cores, and a single biopsy composed of medulla only. Characteristic viral cytopathic changes in the tubular epithelial cells ("PyVAN changes") were seen in 91 (73%) cases, while the remaining 33 (27%) biopsies lacked viral inclusion bodies. In the cases with two or more cores, diagnostic PyVAN changes were limited to one core in 22 (21%) biopsies. PyVAN changes limited to the medulla were seen in 5 (4%) cases. Polyomavirus load based on the extent of SV40 staining ranged from 0.2% to 80% (median 6%).

Characteristics of PyVAN Classes

The proposed PyVAN classification schema was applied to our study cohort of 124 cases [11], resulting in class 1, $n = 22$ (18%); class 2, $n = 84$ (68%); and class 3, $n = 18$ (14%). The summary of the pvl and ci scores is provided in Table 3. Analysis of the remaining Banff scores (other than interstitial fibrosis which is one of the scores defining the classes) showed significant differences between the classes for total inflammation (ti; $P < 0.003$) and inflammation associated with interstitial fibrosis and tubular atrophy (i-IFTA; $P < 0.003$). Both were the lowest in class 1 ($P < 0.003$) and the highest in class 3 ($P < 0.003$). There was no significant correlation between the classes and the scores for inflammation (i), tubulitis (t), vasculitis (v), glomerulitis (g), peritubular capillaritis (ptc), transplant glomerulopathy (cg), mesangial expansion (mm), arterial hyalinosis (ah and aah), and arteriosclerosis (cv)."

Table 3. Pathologic characteristics of PyVAN.

Pathologic parameters defining PyVAN	Class 1 *N* = 22	Class 2 *N* = 84	Class 3 *N* = 18
PVL scores *n* (%)			
pvl 1	22 (100)	4 (5)	0 (0)
pvl 2	0 (0)	53 (63)	0 (0)
pvl 3	0 (0)	27 (32)	18 (100)
Ci scores *n/N* (%)			
ci 0	6 (27)	23 (27)	0 (0)
ci 1	16 (73)	36 (43)	0 (0)
ci 2	0 (0)	20 (24)	12 (67)
ci 3	0 (0)	5 (6)	6 (33)

ci, Banff interstitial fibrosis scoreN, number of cases in the class, n, number of cases for given measured parameter, pvl, polyomavirus load; PyVAN, polyomavirus nephropathy.

Clinical parameters according to class are presented in Table 4. The diagnoses of PyVAN in classes 1, 2, and 3 were established at medians of 22, 28, and 54 weeks after transplant, respectively. The time of the diagnosis of class 3 was significantly longer after transplant compared with class 1 ($P = 0.002$) and class 2 ($P = 0.033$). The difference in the time of PyVAN diagnosis between classes 1 and 2 was not significant. Neither the mean of plasma BK PCR nor the time of the index biopsy or the highest (peak) plasma BK PCR readings were statistically different between the classes. The median of baseline S-Cr of all the patients was 1.5 mg/dl (IQR 1.2–1.9 mg/dl), and it was similar across the three classes.

At the time of the index biopsy, the mean S-Cr of all patients increased to 2.1 mg/dl (IQR 1.7–2.8 mg/dl) and was not statistically significant across the classes. Similarly, neither the change (in mg/dl) of S-Cr from baseline to peak nor the percentage of patients with less than 15% change from baseline to peak differed among the classes. The percent change in S-Cr from baseline to peak differed significantly between classes 1 and 2 ($P = 0.041$). At baseline, the median of all patients' eGFR was 51 ml/min/1.73^2 (IQR 38.75–66 ml/min/1.73^2) and was the highest in class 1.

eGFR at the time of PyVAN diagnosis was similar across the classes with all showing decrease from baseline. The absolute change in eGFR was the largest in class 1, significantly greater than the decrease in class 2, but percent change was not different among the groups.

Hematuria was more commonly seen in patients with classes 1 and 2 than in class 3 (40%, 16%, and 6%, respectively). Similarly, proteinuria was more frequent in patients with class 1 (61%) than in class 2 (32%) or class 3 (41%).

Allograft status at 24-month follow-up

By study design, all patients had follow-up data for ≥ 24 months after the index biopsy. Our outcome analysis was focused on allograft function, the incidence of graft loss, and PyVAN resolution at follow-up.

Allograft function

All classes showed decline in allograft function during the 24 months from the index biopsy. The increased S-Cr levels observed at the time of PyVAN diagnosis did not resolve back to baseline, but rather continued to rise (Fig. 1). At the time of follow-up, the patients in class 3 had the highest level of serum creatinine level (geometric mean 3.23 mg/dl) when compared to classes

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Table 4. Clinical parameters related to PyVAN.

Clinical parameters	PyVAN Class 1	Class 2	Class 3	*P* Value
Time between transplant and PyVAN diagnosis, wk				
Median	22a	28_a	54_b	0.003
IQR	15–36	18–65	33–94	$C_1C_3 = 0.002$
N	22	84	18	$C_2C_3 = 0.033$
Plasma BK PCR readings ($\times 10^4$)				
Median	6.20	13.09	9.90	0.127
IQR	0.67–8.67	3.41–34.47	2.53–60.64	
N	21	80	17	
Plasma peak BK PCR readings ($\times 10^4$)				
Median	9.68	21.81	9.90	0.747
IQR	5.83–44.50	5.20–89.35	4.70–76.18	
N	21	80	17	
Baseline S-cr within 4 mo before index bx, mg/dl				
Median	1.35	1.60	1.50	0.178
IQR	1.10–1.79	1.30–1.95	1.20–2.20	
N	22	84	18	
Peak S-cr at the index biopsy, mg/dl				
Median	1.85	2.20	2.20	0.704
IQR	1.70–2.90	1.62–2.70	1.90–3.02	
N	22	84	18	
Change in S-Cr, mg/dl baseline to peak, mg/dl				
Median	0.70	0.51	0.75	0.194
IQR	0.4–1.2	0.30–0.90	0.30–1.10	
N	22	84	18	
% Change in S-Cr baseline to peak				0.028
Median	56.44_a	33.23_b	40.04	$C_1C_2 = 0.041$
IQR	26.08–100	19.37–60	25–77.77	
N	22	84	18	
Pts with less 15% change in S-Cr baseline to peak				
n/N (%)	1/22 (4.54)	17/84 (20.23)	3/18 (16.66)	0.260
Baseline eGFR, ml/min/1.73m^2				0.043
Median	61_a	47_b	53	$C_1C_2 = 0.036$
IQR	48–76	38–63	37–66	
N	22	84	18	
eGFR at index biopsy, ml/min/1.73m^2				
Median	38	31	32.5	0.500
IQR	27–47	24–43.5	24–43	
N	22	84	18	
Change in eGFR baseline to peak, ml/min/1.73m^2				
Median	-25.5_a	-14.5_b	−19.5	0.016
IQR	−33; −13	−21,5; −8	−30; −9	$C_1C_2 = 0.016$
N	22	84	18	
% Change in eGFR baseline to peak				
Median	−41.51	−30.44	−36.31	0.087
IQR	−54.23; −25	−43.78; −20.25	−50.00; −23.94	
N	22	84	18	

bx, biopsy; IQR, Interquartile range; N, number of cases with available follow-up, n, number of cases for given measured parameter; patients; pts; PyVAN, polyomavirus nephropathy; S-cr, serum creatinine, eGFR, estimated glomerular filtration rate; wk, weeks.

P values for the medians based on the Kruskal–Wallis test (one-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.

a and b are the pairwise comparisons of PyVAN classes, and the significance values have been adjusted by the Bonferroni correction for multiple tests.

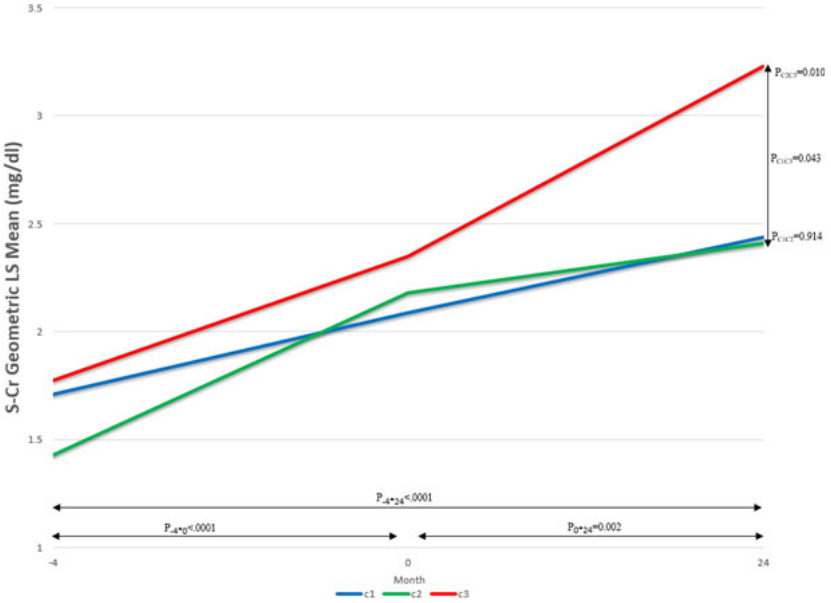

Figure 1 Change in serum creatinine over time for all patients.

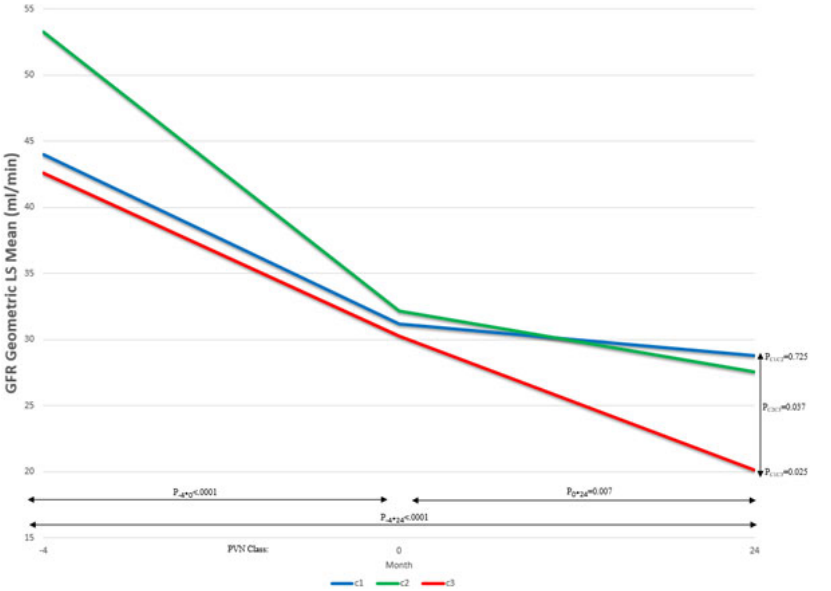

Figure 2 Change in eGFR over time for all patients.

1 and 2, as also mirrored by eGFR changes (Fig. 2). Thus, all classes showed a decline in eGFR from their baseline to the time of the index biopsy ($P < 0.001$) and continued to decrease over follow-up ($P = 0.07$). At 24-month follow-up, the eGFR of patients with class 3 (20.12 ml/min) was significantly lower as compared to class 1 (28.81 ml/min) and class 2 (27.54 ml/min), $P = 0.025$ and $P = 0.037$, respectively. The eGFR levels of classes 1 and 2 were comparable ($P = 0.725$).

Graft failure

We observed that 18 (14.6%) patients experienced graft failure during the first 24 months of follow-up (Table 5). 11 of these patients underwent allograft biopsy, 10 of which showed persistent BKPyVAN, one with concurrent antibody-mediated rejection, and one biopsy showed "chronic allograft nephropathy." Graft failure was not significantly different between the classes

Table 5. Graft failure.

PVN class	Graft failure at 24 mo from index bx (*N* = 124)	Additional graft failure at any time during follow-up (*N* = 106)	All graft failure at any time during follow-up (*N* = 124)
All			
n/N (%)	18/124 (14.61)	12/106 (11.32)	30/124 (24.2)
median (mo)	17.2	46.1	24
Class 1			
n/N (%)	4/22 (18.2)	2/18 (11.11)	6/22 (27.3)
Median (mo)	22.4	53	27.3
Class 2			
n/N (%)	11/84 (13.6)	6/81 (8.21)	17/84 (20.2)
Median (mo)	16.6	40.7	24.0
Class 3			
n/N (%)	3/18 (16.7)	4/15 (26.66)	7/18 (38.88)
Median (mo)	1.9	53	43.6
P value	0.851	0.206	0.468

bx, biopsy, mo, months; N, number of cases with available follow-up, n, number of cases for given measured parameter; Py; VAN, polyomavirus nephropathy.

P value based on the Cochran-Mantel-Haenszel chi-Square test for nonzero Spearman correlation using the midrank scores.

(p=0.851). We did not observe any deaths during the first 2 years of follow-up. Longer duration of follow-up was available for 106 patients and revealed graft failure in 12 (11.3%) additional patients; this measure was not significantly different across the groups. Similarly, overall graft failure at any time of the available follow-up was not significantly different across the classes. In subsequent years, there were 8 deaths, which were not directly related to the allograft dysfunction, including pneumonia (2 cases), cardiac arrest (3 cases), CVA (2 cases), and metastatic cancer (1 case).

PyVAN resolution

At 24 months of follow-up, 62 (52%) patients showed PyVAN resolution by PCR with or without corresponding biopsy. Median time to PyVAN resolution was 28 (IQR 15–47.9) weeks after the diagnostic biopsy (Table 2). The time of observed PCR clearance was not different among classes; 27.1 weeks in class 1, 26.7 weeks in class 2, and 34 weeks in class 3 ($P = 0.703$, Table 6).

Depending on the center, follow-up biopsies were performed in patients with either persistent viremia, elevated serum creatinine, or the presence of new donor-specific antibodies. During the 24-month follow-up period, 65 of 124 (52%) patients had a follow-up biopsy: 30 (46%) of these had persistent PyVAN, 8 (12%) had acute T-cell mediated rejection (including one patient with PyVAN and vascular rejection), and one (1.5%) developed antibody-mediated rejection. The follow-up biopsies included 9 cases from class 1, 47 from class 2 and 9 from class 3 (Table 5). The resolution of PyVAN was observed in 33.3%, 59.6%, and 44.4% ($P = 0.639$) in respective classes. The time of the PyVAN resolution as seen in biopsy was 36 weeks in class 1, 44.4 weeks in class 2, and 56 weeks in class 3 ($P = 0.982$).

Analysis of the effects of pvl and ci scores alone (Table 7) showed that both of these parameters independently predicted serum creatinine and eGFR at 24-month follow-up. Additionally, the pvl score alone was predictive for PVN resolution and viral clearance.

Discussion

Our analysis failed to confirm a predictive value of the newly proposed classification of three tiers for the diagnosis of PyVAN as defined by the Banff Working Group in 2018. The scoring system did not show differences among the classes at initial presentation or predict outcome at 24-month follow-up. We observed that after 24 months of follow-up classes 1 and 2 had a similar outcome as defined by the S-Cr and eGFR, and together, these had better outcome than class 3. A parallel pattern was observed in another study including 50 cases of PyVAN, in which there were no significant

Table 6. PyVAN resolution during 24 months postindex biopsy.

	PyVAN			
	Class 1	Class 2	Class 3	
PyVAN resolution	22	84	18	*P* Value
By biopsy				
n/N (%)	3/9 (33.33)	28/47(59.57)	4/9 (44.44)	0.639
Total number of *N* = 65				
By biopsy or plasma PCR				
n/N (%)	14/21(66.67)	40/80(50)	8/18(44.44)	0.156
N = 119				
Time to resolution by bx, weeks				
Median	36	44.4	56.9	0.982
IQR	30.7–91.6	21–75.6	29.4–85.4	
N	3	28	4	
Time to PCR clearance, weeks				
Median	27.1	26.7	34.6	0.703
IQR	11.6–47.3	15.7–48.4	18.7–36.6	
N	14	40	8	

bx, biopsy, mo, months; IQR, Interquartile range. (*P* values for PyVAN Resolution based on the Cochran-Mantel-Haenszel chi-Square test for nonzero Spearman's correlation using the midrank scores. *P* values for time to PyVAN resolution based on the Kruskal–Wallis test (one-way analysis of variance of the rank scores); N, number of cases with available follow-up, n, number of cases for given measured parameter; PyVAN, polyomavirus nephropathy.

Table 7. Analysis of outcomes as a function of pvl and ci scores.

Outcome measures	Pvl score effect (*P*-value)	Ci score effect (*P*-value)
S-Cr from baseline to the 24 months	<0.0001*	<0.0001*
GFR from baseline to the 24 months	<0.0001*	<0.0001*
Graft failure at 24 months postbiopsy	0.9365	0.4037
Graft survival at last follow-up	0.5090	0.4684
PyVAN resolution and viral clearance	0.0146*	0.3083

ci, Banff interstitial fibrosis score; pvl, polyomavirus load; PyVAN, polyomavirus nephropathy; S-Cr, serum creatinine.

For effect on pvl and ci on S-Cr and eGFR mixed-model for repeated measurements was used. For the remaining covariates, the analysis of the covariates effect on outcome measures using probit regression was performed.

*Denotes significant values.

differences in the death-censored graft survival between the groups, although the class 3 group in this study numerically had the worst allograft outcome [17]. Also, we were not able to predict either graft loss or PyVAN clearance (by the biopsy or PCR results) using this classification.

In an attempt to reproduce the patient populations used in the initial study and the validation study that included patients from European and US centers, we have used similar inclusion criteria in terms of the age of the patients and the lack of coexisting cell- or antibody-mediated rejection, and collected a similar set of clinical and pathological data. Our cohort had many similarities to that previously published in respect of number of cases, age, gender and race distribution, and source of the graft [11,16].

We observed a striking difference in the timing of the initial biopsy in class 3 when compared to classes 1 and 2. In our study, as well as in the previous studies, the diagnosis of PyVAN class 3 was made much later in the course post-transplant compared with classes 1 and 2 (median 12 months vs. 6 months for classes 1 and 2). This difference suggests that these class 3 cases represent a more chronic stage of PyVAN characterized by the presence of significant fibrosis, which is a defining variable of class 3, and a known complication of PyVAN

[6]. In addition to interstitial fibrosis, class 3 cases had significant inflammation in the areas of interstitial fibrosis and tubular atrophy, which has also been previously recognized as an unfavorable factor [18]. In contrast to previously published work [19], the interstitial inflammation in the nonfibrotic areas and/or tubulitis were not considered in the classification, and when analyzed in our cohort, they did not correlate with the new classes.

Our study also showed that the new classes of PyVAN did not correlate with the level of BK viremia whether at its highest level (peak) or at the time of the biopsy. This observation is in disagreement with studies that showed a correlation of the BK viremia with increased risk of interstitial fibrosis and the subsequent outcome [20,21].

Contrary to the findings of the Banff Working group, in our study neither the S-Cr level nor eGFR at the time of diagnostic renal biopsy were significantly different across the group, and they were not predictive of the outcome at 24-month follow-up [11]. Our results are in agreement with the study by Bouatou *et al.* in which both of these measures were also not significantly different between the classes [17].

The analysis of graft failure in our cohort did not demonstrate correlation with class of PyVAN. Numerically, there was more graft failure at both 24-month and longer follow-up in class 3 compared with classes 1 and 2, but the difference was not significant. These findings are in disagreement with the originally published classification that showed a significant change in graft failure across the classes, which was unchanged whether the patients had other complications postindex biopsy or not [11]. However, the subsequent studies that utilized the proposed classification did not confirm this association [16,17]. Similarly, neither our analysis nor the other previously published data found any impact of the class assignment on PVN resolution (including the timing postdiagnosis).

It appears that in our and the previously published studies, there was an uneven distribution of the classes in the given cohort, with the majority of the cases (68% this study, 63%, [11] 55%, [16] and 62% [17]) classified as class 2, followed by classes 1 and 3. Such skewed distributions could have adversely affected the model's performance, especially with the regression-based models.

One of the limitations of our study might be the fact that it utilized cases from different centers and the scoring of the biopsy findings was done by independent pathologists without central review of the slides. This approach is different to those previously published, but it mimics the day-to-day practice and it stresses the importance that any classification should be applied without major interobserver variability. Another caveat in this and other studies on PyVAN is the role of lowering immunosuppression as a mainstay of management of BKV infection to increase immune response to the virus. This approach, while considered safe, may result in greater alloimmune activity and in some patients may result in rejection and eventually affect the allograft survival despite viral clearance [10,21]. In our study group, we identified as many as 9 patients (13%) who were found to have rejection in the follow-up biopsy.

In conclusion, the proposed classification of PyVAN is promising for the evaluation of allograft biopsies; however, the classes do not stratify and identify patients at increased risk of allograft failure and do not correlate with the previously identified prognostic indicators such interstitial inflammation or viral load. In our cohort, while class 3 was associated with the worst prognosis, there was no statistical difference between classes 1 and 2 in terms of outcome. It appears that the timing post-transplantation and inflammation in IFTA are additional factors contributing to an adverse allograft outcome in PyVAN.

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Conflict of interest

None.

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Authorship

JK designed the study, performed research, collected data, analyzed data, and wrote the paper. APP, MEK, ABF, and DAT collected data and contributed to the writing and approval of the paper. MYL, AM, MD, RS, MC, DDM, and DK collected data and contributed to the approval of the paper. IEM performed statistical analysis and contributed to the writing of the paper. TRM designed the study, collected data, and contributed to the writing and approval of the paper.

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