A Multicenter Application of the 2018 Banff Classification for BK Polyomavirus-associated Nephropathy in Renal Transplantation

Bouatou, Yassine; Nguyen, Tri Q.; Roelofs, Joris J. T. H.; Bemelman, Frederike J.; Michielsen, Laura; Goldschmeding, Roel; Kers, Jesper; Florquin, Sandrine

Published in:
Transplantation

DOI:
10.1097/TP.0000000000002712

Published: 01/01/2019

Document Version
Publisher's PDF, also known as Version of record

Citation for pulished version (APA):
Bouatou, Y., Nguyen, T. Q., Roelofs, J. J. T. H., Bemelman, F. J., Michielsen, L., Goldschmeding, R., Kers, J., & Florquin, S. (2019). A Multicenter Application of the 2018 Banff Classification for BK Polyomavirus-associated Nephropathy in Renal Transplantation. *Transplantation*, *103*(12), 2692-2700. https://doi.org/10.1097/TP.0000000000002712

A Multicenter Application of the 2018 Banff Classification for BK Polyomavirus-associated Nephropathy in Renal Transplantation

Yassine Bouatou, MD,[1,2] Tri Q. Nguyen, MD, PhD,[3] Joris J.T.H. Roelofs, MD, PhD,[1] Frederike J. Bemelman, MD, PhD,[4] Laura Michielsen, MD, PhD,[5] Roel Goldschmeding, MD, PhD,[3] Jesper Kers, MD, PhD,[1,6,7] and Sandrine Florquin, MD, PhD[1]

Background. With current immunosuppressive regimens, BK polyomavirus-associated nephropathy (BKPyVAN) is still a matter of concern. Stratification of patients at risk for allograft loss is of uttermost importance to guide treatment choice and assess prognosis. In 2018, the Banff working group proposed a classification scheme for the prognosis of BKPyVAN, but external application on independent cohorts is yet to be performed. We investigated how the 2018 Banff classification would perform in a multicenter cohort comprising 50 cases of biopsy-proven BKPyVAN compared to previously published classification systems. **Methods.** We analyzed consecutive BKPyVAN cases from two Dutch university hospitals between 2002 and 2013, retrieved clinical data, and scored all biopsies according to the Banff 2018 classification, and as a comparison, 4 previously proposed BKPyVAN classification systems. We used estimated glomerular filtration rate trajectories and death-censored graft survival as primary endpoints. **Results.** The 2018 Banff classification did not associate with estimated glomerular filtration rate decline or graft failure and performed only slightly better than the 4 previously proposed classifiers. Anti-human leukocyte antigen donor-specific antibodies (DSAs), especially in combination with ongoing biopsy-proven BKPyVAN on follow-up, did correlate with graft function and survival. Patients who were DSA+/BKPyVAN+ on follow-up had more inflammation at the baseline biopsy, which by itself was not associated with graft outcomes. **Conclusions.** Neither the 2018 Banff BKPyVAN classification nor previously published stratification systems could be applied to our multicenter patient cohort. Our data suggest that there might be a prognostic value for follow-up biopsies and DSA measurements to improve risk stratification after BKPyVAN, although prospective multicenter efforts with protocol measurements are needed to confirm this.

(*Transplantation* 2019;103:2692–2700)

INTRODUCTION

Human BK polyomavirus (BKPyV) is found in urine samples from the general population.[1,2] BKPyV resides in the urothelium and BKPyV-associated nephropathy (BKPyVAN) is observed in 5% to 10% of kidney transplant recipients. It is associated with allograft loss during the first years after transplantation.[3-5] Early detection of BKPyVAN allows appropriate reduction of the immunosuppressive medication, which leads to reversal of disease in about 80% of the cases, while late detection results generally in allograft loss.[6,7]

Received 20 September 2018. Revision received 26 February 2019.

Accepted 1 March 2019.

[1] Amsterdam UMC, University of Amsterdam, Department of Pathology, Amsterdam Infection & Immunity Institute (AI&II), Amsterdam Cardiovascular Sciences (ACS), Meibergdreef 9, Amsterdam, The Netherlands.

[2] Department of Nephrology, Geneva University Hospitals, Geneva, Switzerland.

[3] Department of Pathology, University Medical Center Utrecht, Utrecht, The Netherlands.

[4] Amsterdam UMC, University of Amsterdam, Renal Transplant Unit, Amsterdam Infection & Immunity Institute (AI&II), Meibergdreef 9, Amsterdam, The Netherlands.

[5] Department of Nephrology, University Medical Center Utrecht, Utrecht, The Netherlands.

[6] Center for Analytical Sciences Amsterdam (CASA) - Biomolecular Systems Analytics, Van 't Hoff Institute for Molecular Sciences (HIMS), University of Amsterdam, Amsterdam, The Netherlands.

[7] Ragon Institute of Massachusetts General Hospital, Massachusetts Institute of Technology and Harvard University, Cambridge, MA.

Y.B. was supported by a grant from Geneva University Hospitals. J.K. was supported by a Kolff Junior Postdoc Grant of the Dutch Kidney Foundation (17OKG23) and a Work Visit Grant of the Amsterdam Infection and Immunity Institute (AI&II) of the Amsterdam University Medical Centers.

The authors declare no conflicts of interest.

J.K. and S.F. participated in research design. Y.B., T.Q.N., J.J.T.H.R., F.J.B., L.M., R.G., J.K., and S.F. participated in writing the article. Y.B., T.Q.N., J.J.T.H.R., F.J.B., L.M., R.G., J.K., and S.F. participated in data collection. Y.B., J.K., and S.F. participated in the performance of the research. Y.B., J.K., and S.F. participated in data analysis.

Supplemental digital content (SDC) is available for this article. Direct URL citations appear in the printed text, and links to the digital files are provided in the HTML text of this article on the journal's Web site (www.transplantjournal.com).

Correspondence: Jesper Kers, MD, PhD, Amsterdam UMC, location AMC, Department of Pathology, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands. (j.kers@amc.uva.nl).

ISSN: 0041-1337/19/10312-2692

DOI: 10.1097/TP.0000000000002712

The diagnostic criteria for BKPyVAN have evolved over the past few years. Still, a diagnosis of definitive BKPyVAN can only be made on renal biopsies immunohistochemically stained positive for the viral SV40 large-T-antigen.[8] There is currently an unmet need for parameters that enable clinicians to stratify patients at risk for progressive transplant decline and graft failure. There has been discussion over the past years on the value of biopsy-related parameters and scoring systems that claimed to predict outcome and to date, but none are routinely used in clinical practice to guide the treatment strategy; over-immunosuppression will lead to ongoing viral replication in renal transplant tissue and underimmunosuppression could lead to (re)activation of the allograft response leading to rejection.[9] Besides being obligatory for the diagnosis of definite BKPyVAN, quantification of viral replication with SV40 large-T-antigen staining could also have prognostic value. The Banff Initiative for Quality Assurance in Transplantation (BIFQUIT) working group evaluated the reproducibility of SV40 large-T-antigen in 60 centers with 81 pathologists involved.[10] Given their findings, a simplification of BK immunohistochemical scoring was proposed using a three-tier grading system (pvl0-3) representing the percentage of SV40-stained nuclei (pvl0: none, pvl1: <10%; pvl2: 10% to 25%; pvl3: >25% of tubules with nuclear staining). Also, histopathological parameters found at the time of biopsy could have prognostic value. For this reason, the Banff working group developed a novel classification scheme based on the data from 192 patients transplanted in 9 medical centers from the United States and Europe between 1996 and 2008 who developed biopsy-proven BKPyVAN.[11] The new classification scheme is a combination of the three-tier pvl score and the Banff interstitial fibrosis (ci) score. Interobserver agreement of the score was not determined since both parameters were previously shown to be substantially reproducible. Most importantly, external validation of the 2018 classification has not been performed yet, which limits generalizability and wide-spread application in clinical practice as of yet.

The primary aim of the current study was to apply the latest Banff 2018 classification for BKPyVAN in our independent multicenter cohort comprising 50 biopsy-proven cases and test how well it predicts the prognosis of the renal transplant recipients. Since it is known that the follow-up after biopsy is at least partly determined by weaning of immunosuppression, we additionally determined how donor-specific antibodies (DSAs) and diagnosis on follow-up biopsies were associated with renal allograft outcomes as a secondary outcome.

MATERIALS AND METHODS

Study Design and Patient Population

We retrospectively collected data from 50 consecutive patients with a biopsy-proven BKPyVAN from the Amsterdam University Medical Center (UMC), location Academic Medical Center (N = 35) and the UMC Utrecht (N = 15) meeting the minimal criteria for adequacy of at least 7 glomeruli and 1 artery in the period of 2002 until 2013 assuring at least 4 years of follow-up after the index biopsy for all cases. The first biopsy with a diagnosis of definitive BKPyVAN (SV40 large-T-antigen positive) was chosen for in-depth histological analysis and disease stratification according to the 2018 Banff classifications (hereafter referred to as "baseline biopsy"). No patients were lost to follow-up. The electronic patient database was used to collect all relevant clinical data and biopsy material that was left-over after diagnostics and was used as study material. All information was processed anonymously according to the code of conduct by the Federation of Dutch Medical Scientific Society (FDMSS).[12]

Renal Allograft Recipients

Standard immunosuppressive medication during that time-period consisted of induction therapy with basiliximab (Simulect; Novartis Pharma B.V., The Netherlands), prednisolone, a calcineurin inhibitor; either cyclosporine A (Neoral; Novartis Pharma B.V., The Netherlands) or tacrolimus (Prograf; Astellas Pharma, The Netherlands) and the proliferation inhibitor mycophenolate mofetil (Cellcept; Roche B.V., The Netherlands). Basic immunosuppressive regimens are not different between the two UMCs. BK blood polymerase chain reaction screening was performed every 3 months after transplantation. All patients with BKPyVAN underwent reduction of immunosuppression as follows: mycophenolate mofetil was discontinued, and after 4 weeks when the BK blood polymerase chain reaction stayed above 10 000 copies/mL, calcineurin inhibitor was reduced and administration of intravenous immunoglobulins was administered according to the center's decision.

Histology and (Immuno) Histochemical Stainings

The biopsy material was formalin-fixed and paraffin-embedded. Standard staining consisted of hematoxylin and eosin staining, Jones' silver staining, and periodic acid-Schiff after diastase staining. A monoclonal anti-SV40 large-T-antigen antibody was used (clone PAb416; EMD Millipore, The Netherlands). Biopsies were centrally scored according to the latest Banff updates by 2 observers from one center (S.F. and J.K.) simultaneously.[13,14] Additionally, the total inflammation score (ti-score), which includes inflammatory infiltrates in areas with fibrosis, was evaluated and scored as a percentage of inflammation per 10% of parenchyma. Banff 2009 Classes for BKPyVAN were attributed according to the Banff working proposal[15]: Class A: Variable number of virus infected cells with no or minimal injury to tubular epithelial cells; Class B: Tubular epithelial cell necrosis or lysis with denudation of basement membrane across a length of more than 2 cells; Class C: Any degree of tubular injury with interstitial fibrosis affecting >50% of cortex. The Banff 2009 scheme is a simplified version of the classification as proposed by Drachenberg and colleagues and the American Society of Transplantation.[16,17] The percentage of BKPyV-infected tubules (pvl score) was scored according to the method described in the article by Nickeleit et al.[11] as follows: pvl1: ≤1% positive tubules/ducts, pvl2: 1% to 10% positive tubules/ducts, and pvl3: >10% positive tubules/ducts. Note that this three-tier scoring system is not in accordance with the validated BIFQUIT study.[10] Subsequently, Banff 2018 Classes were scored according to Nickeleit et al[11] as follows: Class 1: pvl-score 1 and ci-score 0 to 1; Class 2: pvl-score 1 and ci-score 2 to 3, pvl-score 2 and ci-score 0

to 3, or pvl-score 3 and ci-score 0 to 1; Class 3: pvl-score 3 and ci-score 2 to 3. All 50 cases were negative for C4d and none of the cases could additionally be classified as C4d-negative antibody-mediated rejection (AMR) on their initial baseline biopsy. None of the included cases had a Banff v-score > 0. BKPyVAN co-occurrence with T cell-mediated rejection (TCMR) grade I cannot be excluded, since no diagnostic criteria exist to distinguish both entities. All of the included cases had tubulointerstitial nephritis in the areas of viral inclusions.

Outcome Measures

We investigated as a primary outcomes death-censored allograft failure, defined as the return to dialysis, retransplantation or an estimated glomerular filtration rate (eGFR) of less than 10 mL/min on at least 2 follow-up time-points without a regain of renal function thereafter. Our secondary outcome was the eGFR trajectory over time. This allowed us to calculate the dynamic association of renal function trajectories over time. We performed log-transformation of the eGFR measures and normal distribution was validated visually. Analysis of the evaluation of graft function over time was performed using linear mixed-effects models.[11] GFRs were estimated from serum creatinine measures using the Modification of Diet in Renal Disease algorithm.[18] From all patients, serum creatinine measures were collected at the time of biopsy (t = 0) and on follow-up at days 7, 30, 60, 90, and 180, and year 1, 2, 3, and 4. In the case of missing data for an individual serum creatinine follow-up, values were imputed (see **Text S1, SDC,** http://links.lww.com/TP/B722). In case of graft failure, an eGFR value of 10 mL/min was imputed for the eGFR trajectories from the time of failure, because we can reasonably assume that renal function does not recover after graft failure.

Predictors

A detailed description of the included predictors, including the intention-to-diagnose imputation approach for DSA measurements can be found Text S1 and Figure S1 (**SDC,** http://links.lww.com/TP/B722).

Statistical Analysis

A detailed description of the statistical approach is described in Text S1 (**SDC,** http://links.lww.com/TP/B722).

RESULTS

Baseline Characteristics of the Kidney Transplant Recipients and Donors

Patients characteristics can be found in Table 1. We did not observe major differences among the three Banff 2018 BKPyVAN Classes. Patients who presented with Banff 2018 BKPyVAN Class 3 were biopsied significantly later after transplantation compared to Banff 2018 Classes 1 and 2: median 533 days (interquartile range [IQR] 368–828) as compared to 146 days (IQR 141–202) and 222 days (IQR 118–615) for Classes 1 and 2, respectively (P = 0.05). The total follow-up time was at a median of 59 months (IQR 37–93 mo) and the follow-up time after the biopsy diagnosis was a median of 41 months (IQR 17–73 mo). At the end of the follow-up period, 20 of 50 (40%) had lost their allograft. For all the 50 patients, the baseline biopsy was the first presentation of acute graft failure and, therefore, none of those patients had a prior episode of biopsy-proven rejection. No significant differences in baseline parameters were observed between the Academic Medial Center and UMC Utrecht cohorts (**Table S1, SDC,** http://links.lww.com/TP/B722). Baseline Banff scores are depicted in Figure S2 (**SDC,** http://links.lww.com/TP/B722).

TABLE 1.

Demographic, clinical, transplantation, and biopsy characteristics of patients with BKPyVAN as baseline

		Banff 2018 classification		
	All	Class 1	Class 2	Class 3
N	50	5	31	14
Donor				
Donation type, N, %				
Living donation	20 (40%)	2 (40%)	13 (42%)	5 (36%)
DCD	9 (18%)	2 (40%)	3 (10%)	4 (29%)
DBD	21 (42%)	1 (20%)	15 (48%)	5 (36%)
Recipient				
Age, median y, IQR	52 (41–61)	54 (40–63)	52 (41–60)	51 (44–64)
Gender, N male, %	27 (54%)	2 (40%)	18 (58%)	7 (50%)
Paediatric recipients, N, %	7 (14%)	1 (20%)	4 (13%)	2 (14%)
Transplantation				
HLA A/B/DR mismatch, median, IQR	3 (2–4)	3 (2–4)	3 (2–4)	3 (2–5)
Cold ischemia time, median min, IQR	14 (2–19)	11 (3–16)	12 (2–19)	17 (3–21)
Delayed graft function, N, %	12 (24%)	0 (0%)	7 (23%)	5 (36%)
At the time of biopsy				
Time of Bx after Tx, median days, IQR	310 (141–668)	146 (141–202)[a]	222 (118–615)[a]	533 (368–828)[a]
eGFR–MDRD, median mL/min per 1.73 m^2, IQR	26 (18–35)	35 (22–42)	25 (18–35)	27 (20–33)

[a]P = 0.05.

BKPyVAN, BK polyomavirus-associated neuropathy; Bx, biopsy; DBD, donation after brain death; DCD, donation after circulatory death; eGFR, estimated glomerular filtration rate; HLA, human leukocyte antigen; IQR, interquartile range; MDRD, modification of diet in renal disease; Tx, Transplantation.

Renal Function Trajectories and Graft Survival According to BKPyVAN Classifications

At baseline, there were no significant difference in the eGFR between Banff 2018 Classes 1 to 3 ($P > 0.15$), Figure 1A. On follow-up, the eGFR trajectories for all 3 Classes declined to a similar extent for the first 500 days, whereas after that they all stabilized without significant differences among the Classes and widely overlapping 95% confidence intervals (CIs) ($P > 0.09$). Numerically, Class 3 had the worst renal function at the end of follow-up, but renal function was also the worst at the time of biopsy. A similar pattern was observed in the survival analysis, where there were no significant differences in death-censored graft survival curves between groups ($P = 0.51$), although again numerically, patients designated Class 3 had the worst allograft outcome (Figure 1B). When we restricted our Kaplan–Meier analysis to the first 24 months follow-up after biopsy, the same results were obtained ($P = 0.51$, data not shown). In Figure S3 (SDC, http://links.lww.com/TP/B722), we compared the results from the Banff 2018 classification with previously published BKPyVAN prognostic classifiers (University of Maryland 2001,[16,19] Polyomavirus Interdisciplinary Work Group 2005,[20] Banff working draft 2009[15] and American Society of Transplantation 2013[17]). Although there were significant between-Class differences in terms of eGFR trajectories for all 4 previously published classifiers, these as well did not follow the expected pattern of A < B (1 to 3) < C from best to worst prognosis in our cohorts. No significant differences were found for the corresponding survival analyses. Including the Banff 2018 classification, we found that 19 of the 50 (38%) were given the same Class in all 5 classifiers (**Figure S4, SDC,** http://links.lww.com/TP/B722).

Associations of Isolated BKPyVAN-related Banff Components With eGFR Trajectories and Graft Survival

In Figure S5 (**SDC,** http://links.lww.com/TP/B722), we further validated whether individual BKPyVAN-related Banff parameters were associated with death-censored graft failure or the eGFR trajectories. The Banff i-score did not associate with death-censored graft failure ($P = 0.99$), but eGFR trajectories differed per group. Compared to patients with <25% interstitial inflammation in nonscarred areas as a reference group, we observed a significantly more declining eGFR trajectory over time for patients with 25% to 50% interstitial inflammation ($P = 0.0004$), but a more stable eGFR trajectory for patients with >50% interstitial inflammation ($P = 0.007$). A similarly complex association with graft outcome was observed for the ti-score. The association between BKPyV-infected tubules (pvl-score) and graft outcome was also complex. We observed that patients with pvl-score 2 had a significantly better graft survival compared to patients with pvl-scores 1 and 3 ($P = 0.041$), which was also reflected by the most stable eGFR trajectories ($P = 0.0003$). There were no significant differences between patients with <25%, 25% to 50%, or >50% interstitial fibrosis and tubular atrophy for death-censored graft failure ($P = 0.64$). The group of patients with <25% ($P = 0.65$) and 25% to 50% interstitial fibrosis and tubular atrophy ($P = 0.01$) had the worst eGFR trajectory over time, whereas the group of patients with >50% interstitial fibrosis and tubular atrophy had relatively stable eGFR trajectories.

DSAs on Follow-up Determine Graft Outcome

Seven of the 50 patients (14%) were DSA-positive at the time of biopsy. None of the 50 patients could concurrently be classified as either C4d-positive AMR (since all cases were per definition C4d-negative) or C4d-negative AMR at baseline. On follow-up, 15 of the 50 patients (30%) developed de novo DSAs, coming to a total of 22 of the 50 patients (44%) with detectable DSAs on follow-up. The presence of DSAs on follow-up (either already present at biopsy or de novo), but not at time of biopsy alone, was associated with significantly worse graft survival in the intention-to-diagnose analysis (log-rank test $P = 0.0026$ versus $P = 0.84$, respectively; Figure 2). We obtained similar results in complete case (**Figure S5, SDC,** http://links.lww.

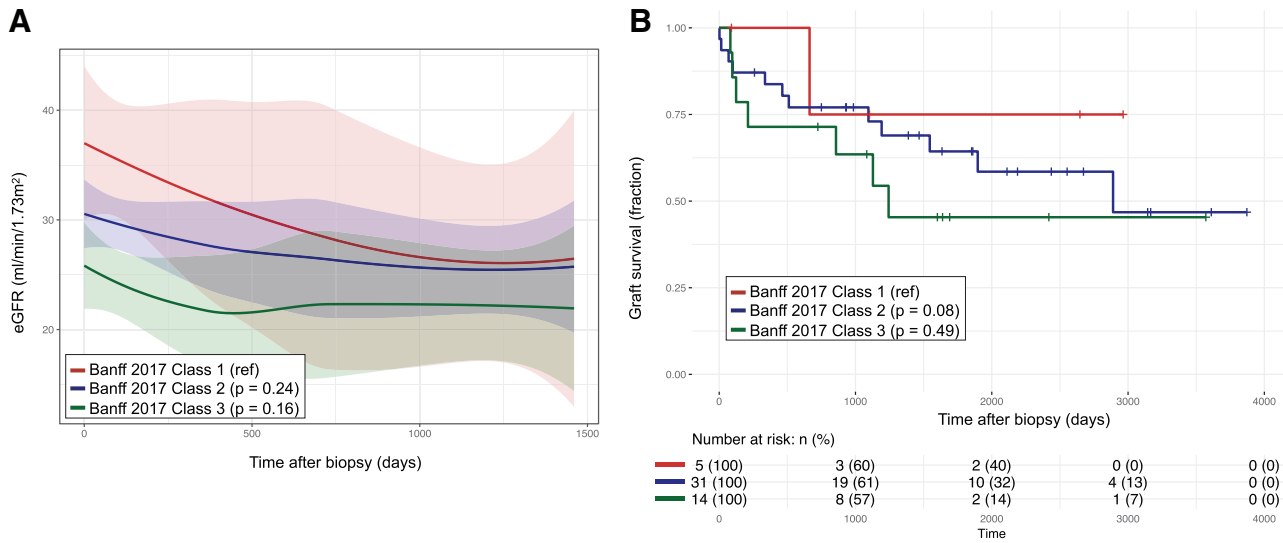

FIGURE 1. Graft outcome for the Banff 2018 BKPyVAN classification. eGFR trajectories (A) and death-censored graft survival curves for (B) the Banff 2018 BKPyVAN classification. When we restricted the analysis to a 24 mo follow-up by right-censoring the data thereafter (in accordance with the Banff 2018 article), the results were the same: $P > 0.44$ for log-transformed linear mixed-effects model on eGFR trajectories and $P > 0.97$ for Kaplan–Meier survival analysis (cumulative incidence of graft failure within 24 mo = 14%). BKPyVAN, BK polyomavirus-associated nephropathy; eGFR, estimated glomerular filtration rate.

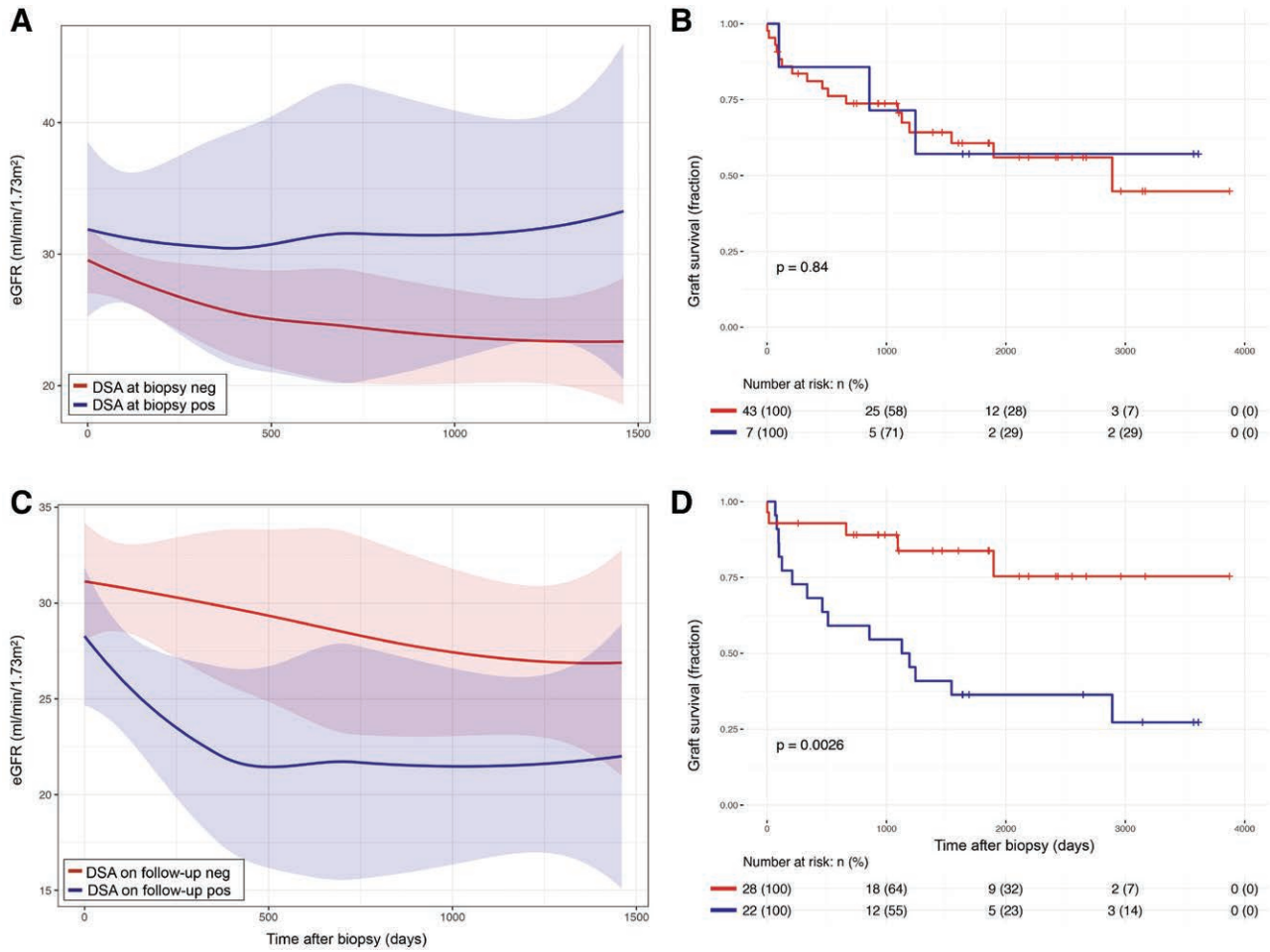

FIGURE 2. Graft outcome for BKPyVAN patients with donor-specific antibodies. eGFR trajectories and death-censored graft survival curves for BKPyVAN patients with DSAs at the time of biopsy (A, B) and DSAs on follow-up after biopsy (C, D). The total number of DSA-positive patients completely overlaps with the patients who were only DSA class II positive on follow-up. Sensitivity analyses with complete case and multivariate imputations with chained equations revealed similar results. BKPyVAN, BK polyomavirus-associated nephropathy; DSA, donor-specific antibodies; eGFR, estimated glomerular filtration rate.

com/TP/B722) and multiple imputations with chained equations (MICE) analyses, even after sensitivity analysis for nonmissing at random (see statistical methods section in **Text S1 and Table S2, SDC,** http://links.lww.com/TP/B722). The eGFR trajectories did not significantly differ between patients who were DSA-positive or DSA-negative at the time of biopsy ($P = 0.30$) or on follow-up ($P = 0.23$), although for the latter analysis, there was a significantly decreasing eGFR trajectory within the first year after baseline biopsy in the group of patients that were DSA-positive on follow-up ($P = 0.0001$). Again, a similar pattern was observed with complete case and MICE analyses, even after additional sensitivity analysis for detection bias. We observed similar results for DSAs directed against human leukocyte antigen (HLA) class I and class II (**Figure S6, SDC,** http://links.lww.com/TP/B722). With respect to the 2018 Banff classification for BKPyVAN at baseline, we observed that 1 of 5 (20%) in Class 1, 11 of 31 (35%) in Class 2, and 10 of 14 (71%) of patients in Class 3 were classified as DSA-positive on follow-up ($P = 0.046$; Figure 3).

Ongoing Biopsy-proven BKPyVAN also Determines Graft Outcome

On follow-up, 21 of 50 patients (42%) had one or more episodes of acute graft dysfunction that required a new renal transplant biopsy. The majority of cases had an ongoing biopsy-proven BKPyVAN (ongoing BKPyVAN; 12/21; 57%), but we also identified patients who underwent rejection after BKPyVAN at baseline (TCMR-only 29%,

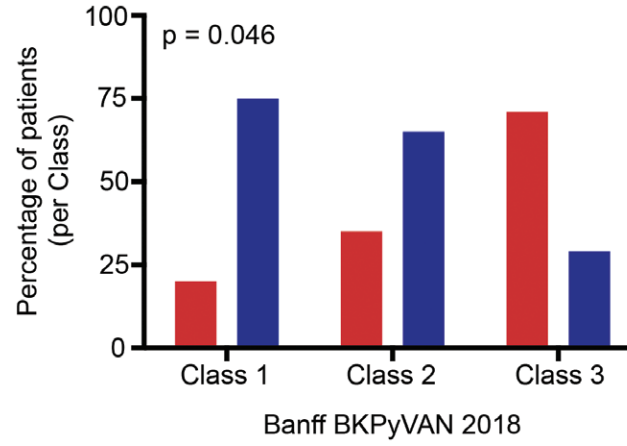

FIGURE 3. The 2018 Banff classification for BKPyVAN correlates with DSA-positivity on follow-up. Patient distribution for follow-up DSA-positivity per Banff 2018 BKPyVAN Class at baseline. BKPyVAN, BK polyomavirus-associated nephropathy; DSA, donor-specific antibodies.

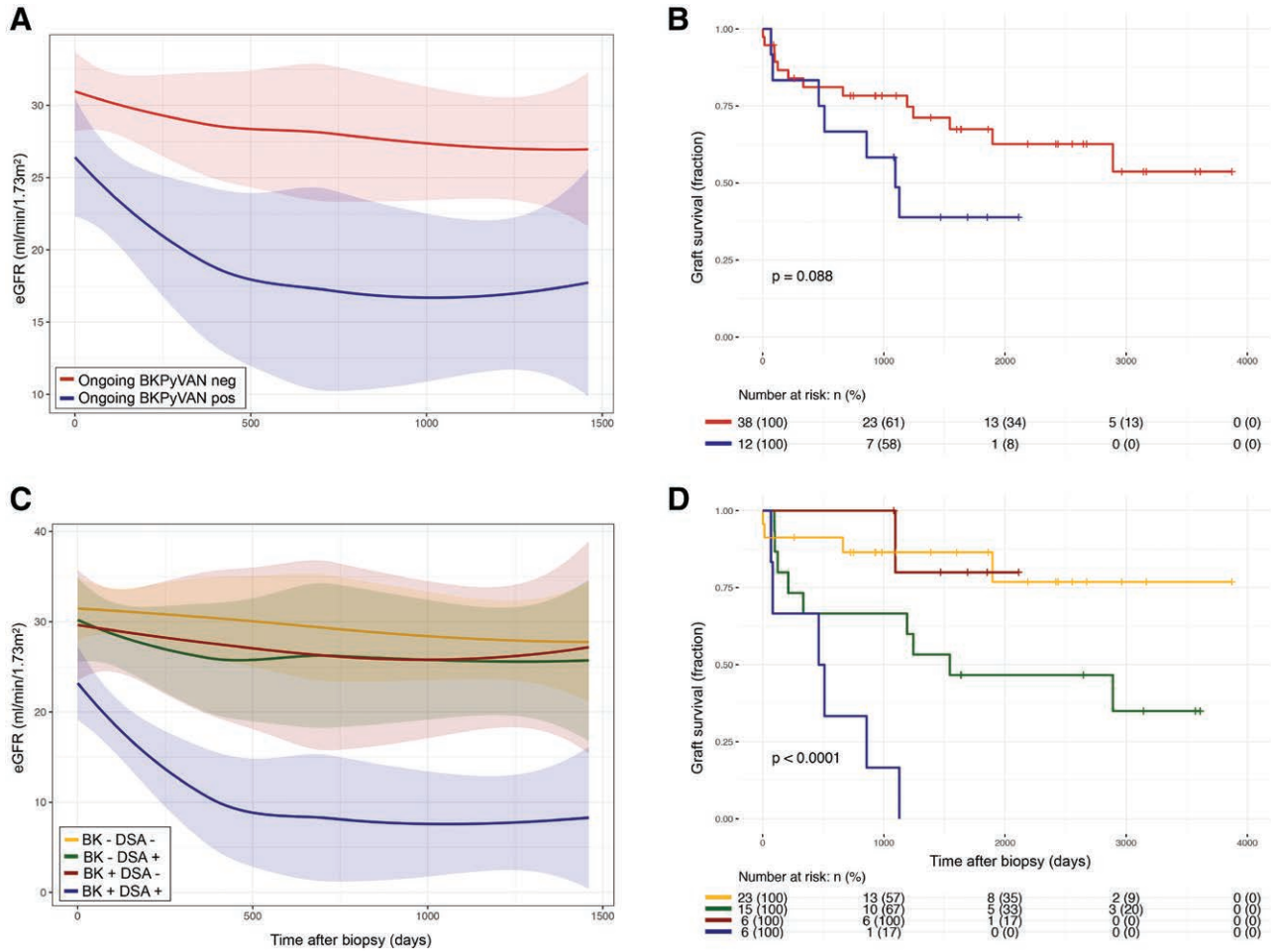

FIGURE 4. Graft outcome for patients who had ongoing biopsy-proven BKPyVAN and DSA on follow-up. eGFR trajectories (A) and death-censored graft survival curves for (B) patients with ongoing biopsy-proven BKPyVAN and patients who had ongoing BKPyVAN and/or DSAs on follow-up after the initial biopsy diagnosis (C, D). Having an episode of graft failure requiring a transplant biopsy, independent of biopsy diagnosis, associated with death-censored graft failure to a lesser extent compared to the subset of patients who had ongoing biopsy-proven BKPyVAN and follow-up DSA-positivity (HR = 2.66, 95% CI = 1.06-6.69, $P = 0.04$ vs HR = 12.52, 95% CI = 3.30-47.43, $P = 0.0002$). BKPyVAN, BK polyomavirus-associated nephropathy; CI, confidence interval; DSA, donor-specific antibodies; eGFR, estimated glomerular filtration rate; HR, hazard ratio.

AMR-only 0%, mixed rejection 14%). In total, 12 of the 50 patients (24%) had a follow-up of ongoing biopsy-proven BKPyVAN. Patients with ongoing biopsy-proven BKPyVAN had a significantly worsening eGFR trajectory after biopsy ($P = 0.004$), whereas the eGFR was not significantly different at the time of biopsy between groups ($P = 0.27$, Figure 4). These patients also showed a trend toward more death-censored graft failure ($P = 0.088$). For rejection on follow-up biopsies (TCMR and mixed rejections), we observed the same trend, but also in the group of patients without rejection, there was a decline in renal function and graft loss was observed (**Figure S7, SDC,** http://links.lww.com/TP/B722). Follow-up rejection and follow-up DSA were not significantly correlated ($P = 0.20$); neither were ongoing BK on follow-up and DSA on follow-up ($P = 0.32$) and ongoing BK virus nephropathy on the follow-up and rejection on follow-up ($P = 0.71$). Also, the sole presence of any follow-up biopsy did not associate with follow-up DSA-positivity ($P = 0.15$). Patients who had a biopsy-proven ongoing BKPyVAN and were also DSA-positive on follow-up (BKPyVAN+/DSA+; 6/50; 12%) had the worst renal function trajectories, leading to an eGFR < 10 mL/min within 500 days after the initial biopsy in most cases ($P < 0.0001$; Figure 4). The other three groups (BKPyVAN-/DSA–, BKPyVAN+/DSA–, and BKPyVAN–/DSA+) had overlapping renal function trajectories. In survival analysis, BKPyVAN+/DSA+ patients also did worst, and all (n = 6) lost their allograft within 1200 days after the initial biopsy ($P < 0.0001$, Figure 4). The hazard ratio of 12.52 for these 6 patients (95% CI 3.30-47.43; $P = 0.0002$) was much higher than the "background" hazard ratio of 2.66 (95% CI 1.06-6.69; $P = 0.04$) of having undergone DSA screening at all. The BKPyVAN–/DSA+ group had the second worst outcome. Both groups that did not have DSAs on follow-up had overlapping survival curves.

Determinants of Ongoing BKPyVAN and DSAs on Follow-up

We made an attempt to see which of the clinical and histopathological parameters differed among the 4 groups as defined by ongoing biopsy-proven BKPyVAN status and follow-up DSA status. Figure 5A displays a heat map with clinical and histological parameters per individual patient within the 4 groups. Figure 5B is the summary of Figure 5A, showing the median values per group as well as boxplots for the ti-score, the factor that discriminated between the 4

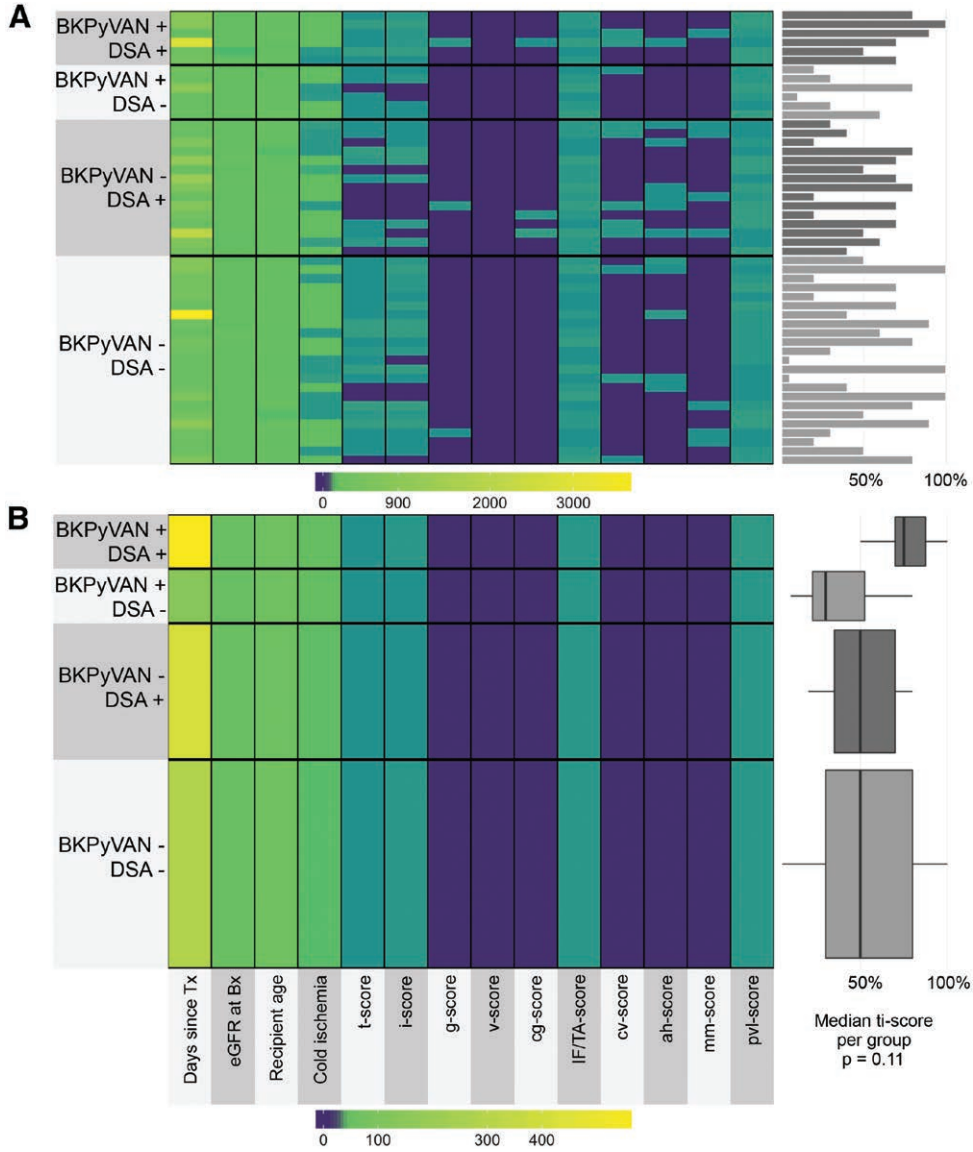

FIGURE 5. Heatmaps of clinical and histopathological data distribution in patients with ongoing biopsy-proven BKPyVAN and DSA. A, Heatmap with individual patient data distribution of clinical and histopathological parameters in patients with ongoing BKPyVAN and/or DSAs on follow-up after the initial biopsy diagnosis. Heatmap (B) is a summary graph depicting median scores for all parameters per diagnostic group. These data indicate that patients with both ongoing BKPyVAN and DSAs on follow-up were diagnosed later after transplantation and had higher Banff ti-scores in the initial BKPyVAN biopsy. BKPyVAN, BK polyomavirus-associated nephropathy; DSA, donor-specific antibodies; ti-scores, total inflammation scores.

groups the best. The patients with persisting biopsy-proven BKPyVAN and DSA-positivity on follow-up (n = 6, 12%) underwent their baseline episode of BKPyVAN relatively late after transplantation (median 576 days, IQR 273–828 d, range 54–2874 d, P = 0.26 for trend over groups, P = 0.29 versus all other groups) and presented with the highest ti-scores (median 75%, IQR 70% to 88%, range 50% to 100%, P = 0.11 for trend, P = 0.04 versus all other groups). We did not identify other baseline clinical parameters (recipient age, cold ischemia time, eGFR at biopsy) or histopathological parameters (Banff scores, pvl-score) that differed between groups.

DISCUSSION

In the present study, performed in a cohort of 50 kidney transplant recipients with a definitive diagnosis of BKPyVAN, we showed that neither the novel Banff 2018 BKPyVAN classification nor previously described classification systems correlated well with renal function or allograft survival. We also showed that the individual histological Banff score at the time of BKPyVAN diagnosis were unable to predict outcomes whereas anti-HLA DSAs, follow-up histological diagnosis, and especially the combination of both associated with allograft outcome instead.

Several previous attempts to stratify BKPyVAN based on morphological parameters failed to provide clinically relevant information to clinician as those classifications did not accurately correlate with allograft loss. The predictive value of the 2009 Working group proposal has been previously assessed in 71 biopsies with BKPyVAN.[21] The proportions of partial and complete responses 3 months after the diagnosis of BKPyVAN has shown a higher trend for class A when compared to B and C, but this result did not reach statistical significance. Interestingly, Banff class

A was associated with a higher risk of graft loss when compared with class B. In our cohort, patients from class C remained relatively stable over time in terms of graft function. The Banff Working Proposal 2009 needed to be modified to incorporate the degree of inflammation. However, in a cohort of 192 patients transplanted from 1996 to 2008 with a biopsy-proven diagnosis of BKPyVAN, Nickeleit et al.[11] used a mixed-effects model with repeated measures to predict allograft function trajectories and a logistic regression model to analyze graft failure at 24 months and constructed a classification system that included the Banff ci and pvl scores. Contrary to prior findings by Masutani et al., the inflammation status seemed not associated with outcome in their cohort.[21] Corroborating on the findings by Masutani et al, we did not observe any association between inflammation (i-score and ti-score) and outcome, but we did observe an association of ti-score at baseline with a group of patients who were both DSA-positive on follow-up and had ongoing biopsy-proven BKPyVAN. These data suggest that the cohort-specific association between extent of inflammation during BKPyVAN and outcome is driven by intermediate complications of both viral persistence and allo-immunity. The functional characteristics of the infiltrate leading to both an ongoing antiviral immune response and an allo-immune response are not deciphered and require further investigation, although T cell clones directed against viral and donor HLA epitopes have been described concomitantly in situ.[22] Two recent studies[23,24] and an accompanying editorial[25] discussed the natural history of BKPyVAN. Clearance of viremia often resulted in more tubulointerstitial inflammation and tubulitis suggesting the development of subclinical rejection, most probably due to a reduction in immunosuppression. A study by Dieplinger and colleagues observed a similar finding with the development of DSAs after BKPyV viremia, especially in cases where viremia persisted, which is in line with our findings.[26] In our study, we observed that patients who were DSA-positive on follow-up with ongoing biopsy-proven BKPyVAN had the worst follow-up with respect to eGFR trajectories as well as graft survival. Altogether these findings suggest that there is a complex interplay between antiviral immunity and the allo-immune response. This complex interplay between antiviral and allo-immune responses might be the reason why stratification systems for BKPyVAN have not appeared to be very useful in the past. The study by Nickeleit et al[11] identified the pvl-score and interstitial fibrosis, but not inflammation, as the parameters best associated with outcome, whereas the study by Masutani et al determined the inverse.[21] Striking to us was also the finding that of the 5 classification systems/proposals tested, 38% of patients were classified in exactly the same class for each of the classification systems.

In the broader context on the use of histology to predict outcomes and determine response to treatment in kidney transplantation, two recent studies from the Paris Transplant Group suggest that, rather than using data at the time of treatment, using data 3-month after treatment (eGFR, DSAs, histology) better stratified patients at risk for adverse outcome.[27,28] This was found for TCMR and AMR, and in the current study, we suggest that this might also be the case for BKPyVAN, although studies with standardized collection of DSAs and protocol biopsies need to confirm this finding. In this study, we were limited by the availability of retrospective data. There was no standardization with regards to DSA measurement and we had to rely on data from both complement dependent cytotoxicity (CDC) and the Luminex method. Therefore, we might have missed some cases with DSAs, because of the inferior sensitivity of the CDC method. Also, we observed that patients who had undergone DSA screening (irrespective of outcome), had a worse outcome, which suggests detection bias. We tried to address the validity of our findings by various (conditional) sensitivity analyses, but standardized measurement of DSAs in a prospective fashion is preferable to address the association with graft function and survival. Although data from both centers was almost complete, we had to impute some of the follow-up serum creatinine measurements, and this might have influenced calculation of the estimates in the mixed-effects models. We also have to acknowledge the relatively small sample size of the current study and cannot completely rule out a statistical type II error. Altogether, we found that the novel 2018 Banff classification for BKPyVAN, as well as previous classification systems, could not be successfully applied to our multicenter cohort. The lack of predictive value might come from a complex interplay between ongoing antiviral immunity, levels of immunosuppression, allo-immune responses, and timing of disease onset/decision to biopsy.

REFERENCES

1. Antonsson A, Green AC, Mallitt KA, et al. Prevalence and stability of antibodies to the BK and JC polyomaviruses: a long-term longitudinal study of Australians. *J Gen Virol.* 2010;91(Pt 7):1849–1853.
2. Egli A, Infanti L, Dumoulin A, et al. Prevalence of polyomavirus BK and JC infection and replication in 400 healthy blood donors. *J Infect Dis.* 2009;199:837–846.
3. Ramos E, Drachenberg CB, Papadimitriou JC, et al. Clinical course of polyoma virus nephropathy in 67 renal transplant patients. *J Am Soc Nephrol.* 2002;13:2145–2151.
4. Hirsch HH. Polyomavirus BK nephropathy: a (re-)emerging complication in renal transplantation. *Am J Transplant.* 2002;2:25–30.
5. Naesens M, Kuypers DR, De Vusser K, et al. The histology of kidney transplant failure: a long-term follow-up study. *Transplantation.* 2014;98:427–435.
6. Babel N, Volk HD, Reinke P. BK polyomavirus infection and nephropathy: the virus-immune system interplay. *Nat Rev Nephrol.* 2011;7:399–406.
7. Wadei HM, Rule AD, Lewin M, et al. Kidney transplant function and histological clearance of virus following diagnosis of polyomavirus-associated nephropathy (PVAN). *Am J Transplant.* 2006;6(5 Pt 1):1025–1032.
8. Nickeleit V, Singh HK. Polyomaviruses and disease: is there more to know than viremia and viruria? *Curr Opin Organ Transplant.* 2015;20:348–358.
9. Sawinski D, Forde KA, Trofe-Clark J, et al. Persistent BK viremia does not increase intermediate-term graft loss but is associated with de novo donor-specific antibodies. *J Am Soc Nephrol.* 2015;26:966–975.
10. Adam B, Randhawa P, Chan S, et al. Banff initiative for quality assurance in transplantation (BIFQUIT): reproducibility of polyomavirus immunohistochemistry in kidney allografts. *Am J Transplant.* 2014;14:2137–2147.
11. Nickeleit V, Singh HK, Randhawa P, et al; Banff Working Group on Polyomavirus Nephropathy. The banff working group classification of definitive polyomavirus nephropathy: morphologic definitions and clinical correlations. *J Am Soc Nephrol.* 2018;29:680–693.
12. Federation of Dutch Medical Scientific Societies. *Human tissue and medical research: code of conduct for responsible use (2011).* Available at https://www.federa.org/sites/default/files/images/print_version_code_of_conduct_english.pdf. Accessed October 1, 2016.
13. Haas M, Loupy A, Lefaucheur C, et al. The banff 2017 kidney meeting report: revised diagnostic criteria for chronic active T cell-mediated rejection, antibody-mediated rejection, and prospects

for integrative endpoints for next-generation clinical trials. *Am J Transplant.* 2018;18:293–307.
14. Roufosse C, Simmonds N, Clahsen-van Groningen M, et al. A 2018 reference guide to the banff classification of renal allograft pathology. *Transplantation.* 2018;102:1795–1814.
15. Sis B, Mengel M, Haas M, et al. Banff '09 meeting report: antibody mediated graft deterioration and implementation of banff working groups. *Am J Transplant.* 2010;10:464–471.
16. Drachenberg CB, Papadimitriou JC, Hirsch HH, et al. Histological patterns of polyomavirus nephropathy: correlation with graft outcome and viral load. *Am J Transplant.* 2004;4:2082–2092.
17. Hirsch HH, Randhawa P; AST Infectious Diseases Community of Practice. BK polyomavirus in solid organ transplantation. *Am J Transplant.* 2013;13(Suppl 4):179–188.
18. Masson I, Flamant M, Maillard N, et al. MDRD versus CKD-EPI equation to estimate glomerular filtration rate in kidney transplant recipients. *Transplantation.* 2013;95:1211–1217.
19. Drachenberg RC, Drachenberg CB, Papadimitriou JC, et al. Morphological spectrum of polyoma virus disease in renal allografts: diagnostic accuracy of urine cytology. *Am J Transplant.* 2001;1:373–381.
20. Hirsch HH, Brennan DC, Drachenberg CB, et al. Polyomavirus-associated nephropathy in renal transplantation: interdisciplinary analyses and recommendations. *Transplantation.* 2005;79:1277–1286.
21. Masutani K, Shapiro R, Basu A, et al. The banff 2009 working proposal for polyomavirus nephropathy: a critical evaluation of its utility as a determinant of clinical outcome. *Am J Transplant.* 2012;12:907–918.
22. Zeng G, Huang Y, Huang Y, et al. Antigen-specificity of T cell infiltrates in biopsies with T cell-mediated rejection and BK polyomavirus viremia: analysis by next generation sequencing. *Am J Transplant.* 2016;16:3131–3138.
23. Nankivell BJ, Renthawa J, Sharma RN, et al. BK virus nephropathy: histological evolution by sequential pathology. *Am J Transplant.* 2017;17:2065–2077.
24. Drachenberg CB, Papadimitriou JC, Chaudhry MR, et al. Histological evolution of BK virus-associated nephropathy: importance of integrating clinical and pathological findings. *Am J Transplant.* 2017;17:2078–2091.
25. Mengel M. BK virus nephropathy revisited. *Am J Transplant.* 2017;17:1972–1973.
26. Dieplinger G, Everly MJ, Briley KP, et al. Onset and progression of de novo donor-specific anti-human leukocyte antigen antibodies after BK polyomavirus and preemptive immunosuppression reduction. *Transpl Infect Dis.* 2015;17:848–858.
27. Viglietti D, Loupy A, Aubert O, et al. Dynamic prognostic score to predict kidney allograft survival in patients with antibody-mediated rejection. *J Am Soc Nephrol.* 2018;29:606–619.
28. Bouatou Y, Viglietti D, Pievani D, et al. Response to treatment and long-term outcomes in kidney transplant recipients with acute T cell-mediated rejection. *Am J Transplant*. 2019.