American Journal of Transplantation 2017; 17: 161–172
Wiley Periodicals Inc.

doi: 10.1111/ajt.13880

Pretransplantation Donor–Recipient Pair Seroreactivity Against BK Polyomavirus Predicts Viremia and Nephropathy After Kidney Transplantation

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Kidney transplant donors are not currently implicated in predicting BK polyomavirus (BKPyV) infection in kidney transplant recipients. It has been postulated, however, that BKPyV infection originates from the kidney allograft. Because BKPyV seroreactivity correlates with BKPyV replication and thus might mirror the infectious load, we investigated whether BKPyV seroreactivity of the donor predicts viremia and BKPyV-associated nephropathy (BKPyVAN) in the recipient. In a retrospective cohort of 407 living kidney donor–recipient pairs, pretransplantation donor and recipient sera were tested for BKPyV IgG levels and correlated with the occurrence of recipient BKPyV viremia and BKPyVAN within 1 year after transplantation. Donor BKPyV IgG level was strongly associated with BKPyV viremia and BKPyVAN ($p < 0.001$), whereas recipient BKPyV seroreactivity showed a nonsignificant inverse trend. Pairing of high–BKPyV-seroreactive donors with low-seroreactive recipients resulted in a 10-fold increased risk of BKPyV viremia (hazard ratio 10.1, 95% CI 3.5–29.0, $p < 0.001$). In multivariate analysis, donor BKPyV seroreactivity was the strongest pretransplantation factor associated with viremia ($p < 0.001$) and BKPyVAN ($p = 0.007$). The proportional relationship between donor BKPyV seroreactivity and recipient infection suggests that donor BKPyV seroreactivity reflects the infectious load of the kidney allograft and calls for the use of pretransplantation BKPyV serological testing of (potential) donors and recipients.

Abbreviations: BKPyVAN, BK polyomavirus-associated nephropathy; BKPyV, BK polyomavirus; c/mL, copies per milliliter; CI, confidence interval; CNI, calcineurin inhibitor; ELISA, enzyme-linked immunosorbent assay; GST, glutathione S-transferase; HR, hazard ratio; LUMC, Leiden University Medical Center; MFI, mean fluorescence intensity; MMF, mycophenolate mofetil; n.p., not possible; OD, optical density; PCR, polymerase chain reaction; PRA, panel reactive antibodies; Q, quartile; VLP, viruslike particle; VP1, viral capsid protein 1

Received 03 February 2016, revised 11 May 2016 and accepted for publication 18 May 2016

Introduction

Solid organ transplant recipients require immunosuppression to prevent allograft rejection. This renders recipients vulnerable to exogenous and endogenous viral infections (reactivation). Regarding the latter, the ubiquitous herpes- and polyomaviruses in particular are involved. No method currently exists to reliably predict these infectious complications; therefore, general and frequent blood viral load monitoring of recipients after transplantation is recommended.

BK polyomavirus (BKPyV) causes asymptomatic infection early in life (1,2), reaching a seroprevalence of ≈90% in adults (3,4). After primary infection, BKPyV latently persists in the urothelium and renal tubular cells (5,6), and small amounts of viral progeny can be temporarily detected in urine of 7–55% of healthy persons, depending on the sampling frequency (7–9).

In immunocompromised patients, BKPyV infections can cause manifest disease, such as hemorrhagic cystitis in hematopoietic stem cell transplant recipients and BKPyV-associated nephropathy (BKPyVAN) in kidney transplant

recipients (1,2,10). Reduction of immunosuppressive therapy is the only effective evidence-based treatment to date (11,12).

BKPyV infection is observed in approximately half of kidney transplant recipients by detection of BKPyV DNA in urine (viruria) (10,12–15). In a subset of viruric recipients (15–30% of the total number of recipients), viral DNA is detected in the circulation (viremia); of these viremic recipients, a small proportion (1–10% of all recipients) develops BKPyVAN, ultimately causing allograft failure (10,12–16). Sustained viremia and BKPyV loads $>10^4$ genome copies per milliliter (c/mL) are associated with BKPyVAN development (1,2,10). To identify this subgroup of recipients at risk who require tapering of immunosuppression (14,17), most kidney transplantation centers regularly evaluate recipients currently for detectable BKPyV DNA in blood (10–12,16).

Immunosuppressive treatment with tacrolimus and rejection treatment with prednisolone have been shown to increase the risk of BKPyVAN (2,10,14,18). Despite intensive study, pretransplantation risk factors for BKPyV viremia and BKPyVAN including age, sex, ethnicity, retransplantation, immunosuppressive regimen, ischemia–reperfusion injury, prior acute rejection episodes, corticosteroid therapy, percentage of panel reactive antibodies (PRA), HLA mismatches, blood group incompatibility, underlying conditions and comorbidities have not been identified (2,10,13–15,19,20). A number of studies, however, reported associations between recipient BKPyV infection and pretransplantation BKPyV serostatus (seropositive or seronegative) of kidney transplant donors and recipients (19,21,22). We considered the donor of particular interest in this regard because BKPyV infection in recipients is thought to originate from the kidney allograft (19,23).

Based on previous studies suggesting that BKPyV seroreactivity is associated with BKPyV replication (21,24,25), we hypothesized that the level of donor BKPyV seroreactivity reflects the BKPyV infectious load of the allograft and thereby predicts BKPyV viremia and BKPyVAN in the recipient. To investigate this hypothesis, living kidney allograft donor–recipient pairs were analyzed for BKPyV seroreactivity before transplantation. Measured pretransplantation levels of BKPyV IgG of donors and recipients were correlated with the incidence of BKPyV viremia and BKPyVAN and compared with other potentially relevant baseline donor, recipient and transplant-related characteristics.

Materials and Methods

Study population and sample collection

To ensure availability of pretransplantation donor and recipient sera, only living kidney allograft donor–recipient pairs were included. All adult (aged >18 years) living donor–recipient pairs transplanted at the Leiden University Medical Center (LUMC) between 2003 and 2013 were eligible for this retrospective cohort study. In total, 519 living donor–recipient pairs were identified. Fifty-three pairs were excluded because no baseline serum sample was available from either donor or recipient; another 59 were excluded because less than two recipient plasma samples collected after transplantation were available for analysis (Figure S1). The remaining 407 donor–recipient pairs were included in the study.

Baseline donor and recipient sera were collected at a mean period of 5.5 mo (range 0.7–26.8 mo) and 0.2 mo (range 0–3.7 mo), respectively, before transplantation. Recipient plasmas screened for BKPyV DNA were collected at five regular time points after transplantation (Figure 1). The mean follow-up was 9.1 mo, and 80%, 95%, 87%, 63%, and 36% of the recipient serum samples were available at time points 1, 2, 3, 4, and 5, respectively. The median number of time points analyzed per recipient was 3.6. All samples were originally collected for routine serological and molecular virus screening and stored at −20°C. The study protocol was submitted to the medical ethics committee of the LUMC, which decided formal approval was not needed because of the retrospective study design and the use of previously collected and anonymized samples.

Detection of BKPyV viremia and assessment of BKPyVAN

To measure the presence of BKPyV DNA in blood, blood plasma was analyzed by quantitative BKPyV real-time polymerase chain reaction (PCR). Using the primers 440BKVs 5′-GAAAAGGAGAGTGTCCAGGG-3′ and 441BKVas 5′-GAACTTCTACTCCTCCTTTTATTAGT-3′ and a TaqMan (Thermo Fisher Scientific, Waltham, MA) probe 576BKV FAM-5′-CCAAAAAGCCAAAGGAACCC-3′-BHQ1, a 90–base pair fragment within the BKPyV viral capsid protein 1 (*VP1*) gene is amplified. Simultaneous isolation, amplification and detection of a standard amount of phocid herpesvirus were used for internal control of inhibition (26).

Routine recipient BKPyV load screening at 1.5, 3 and 6 mo after transplantation was implemented in May 2007. In case of clinical suspicion of BKPyV infection, BKPyV loads were also determined >6 mo after transplantation. In samples obtained before and after 2007 that had not been routinely analyzed, BKPyV loads were determined in retrospect.

Sustained BKPyV viremia was defined as two or more consecutive BKPyV-positive samples spanning ≥3 weeks. Peak viral load was defined as the highest BKPyV DNA plasma load measured in a viremic participant during follow-up.

A kidney biopsy was performed if clinically indicated in the view of the treating physician. BKPyVAN was diagnosed based on immunohistological examination of allograft biopsy specimens showing characteristic pathological features, such as intranuclear viral inclusions in tubular epithelial cells, cell enlargement with polymorphic nuclei, interstitial inflammation and tubular atrophy or fibrosis. BKPyVAN diagnosis was confirmed by immunohistochemical staining with a polyomavirus–cross-reacting mouse monoclonal antibody (PAb416, Calbiochem; EMD Millipore, Billerica, MA) raised against large T antigen of SV40 polyomavirus.

BKPyV serology

Pretransplantation serum samples obtained from 407 donor–recipient pairs, 814 participants in total, were analyzed by an in-house Luminex (Austin, TX) immunoassay detecting IgG reactivity against the BKPyV genotype Ib1 major VP1, according to a published protocol (4,27). This protocol has been used to analyze seroresponses against various human polyomaviruses (4). In brief, 1:100 diluted serum samples were mixed with affinity-purified glutathione S-transferase (GST) BKPyV VP1 fusion protein or with GST alone coupled with fluorescent, unique, colored polystyrene beads (Bio-Rad Laboratories, Hercules, CA). VP1-bound antibodies were detected with biotinylated goat antihuman IgG (H+L; Jackson

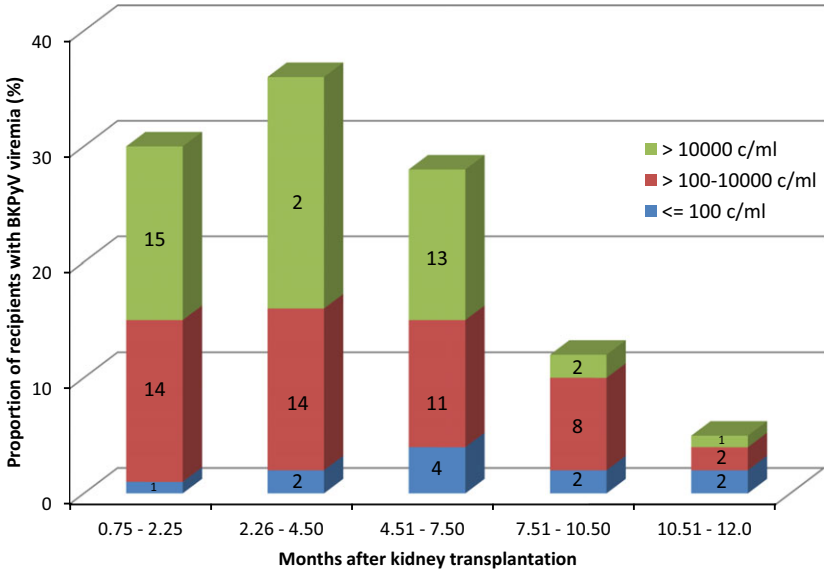

Figure 1: Characteristics of BKPyV viremia in viremic recipients (n = 111). The time points (ranges) of first detection of BKPyV viremia after kidney transplantation are indicated in months after transplantation, and the levels of the measured peak BKPyV loads are shown in copies per milliliter. BKPyV, BK polyomavirus; c/mL, copies per milliliter.

ImmunoResearch, West Grove, PA) and streptavidin-R-phycoerythrin (Invitrogen, Carlsbad, CA). The bead colors and the phycoerythrin signal were analyzed in a Bio-Plex 100 analyzer (Bio-Rad) and expressed as mean fluorescence intensity (MFI). MFI values obtained with GST alone were subtracted to obtain BKPyV VP1-specific signals. The cutoff value to determine BKPyV seropositivity was based on sera of healthy children aged 10–15 mo, as described (4,27).

A serially diluted control serum was included on each plate to control for interplate (n = 10) test variance. High agreement was observed between the test plates ($r = 0.963–0.999$, $p < 0.001$). Good intertest reproducibility of the assay was previously shown for trichodysplasia spinulosa–associated polyomavirus in a group of 80 kidney transplant recipients (28) and was calculated in these recipients for BKPyV ($r = 0.891$, $p < 0.001$).

Of the 407 donor samples included in the current study, 396 (97.3%) were independently reanalyzed for serological confirmation with BKPyV VP1 virus-like particles by enzyme-linked immunosorbent assay (ELISA), as described previously (29–31). The VP1 antigen of this assay was obtained from BKPyV genotype Ib2 (29–31), which differs by five amino acids from Ib1 (data not shown).

Immunosuppression, rejection treatment, and management of BKPyV infection

Induction treatment consisted of basiliximab (93%) or alemtuzumab (7%), and the standard maintenance immunosuppressive regimen included a calcineurin inhibitor (CNI), tacrolimus (76%) or cyclosporin A (24%), combined with corticosteroids (100%) and mycophenolate mofetil (MMF; 99.5%), azathioprine (0.25%), or everolimus (0.25%).

The targeted 12-h area under the curve of the CNI in the first weeks after transplantation was 160–200 μg · h/L for tacrolimus and 4500–5500 μg · h/L for cyclosporin A. The dose of the CNI was tapered 6 weeks after transplantation to targeted 12-h areas under the curve of 80–100 and 3000–3500 μg · h/L, respectively. Rejection treatment consisted of methylprednisolone 1000 mg intravenously once daily for 3 days.

In case of a positive BKPyV load, since 2007, a monthly screening interval was implemented until the BKPyV PCR was negative. In case of a BKPyV load $<10^4$ c/mL, MMF was reduced by 50% and CNI serum levels were evaluated and, if needed, adjusted accordingly. If tacrolimus was used, prednisolone was lowered to 5 mg/day, and in the case of cyclosporin A, prednisolone was lowered to 7.5 mg/day. Detection of a BKPyV load $\geq 10^4$ c/mL prompted adjustment of the immunosuppressive regimen by 50% reduction of the CNI, reduction of mammalian target of rapamycin inhibitor, and 50% reduction or cessation of MMF.

Statistical analyses

Data were analyzed with IBM SPSS Statistics software version 20 (IBM Corp, Armonk, NY). Descriptive analyses were used to report cohort characteristics. Differences between viremic and nonviremic recipients and viremic recipients with or without BKPyVAN were assessed using the chi-square test, Fisher exact test, Student t-test or Mann–Whitney *U* test, as appropriate. To indicate onset of recipient BKPyV viremia, separate Kaplan–Meier curves were generated according to donor and recipient BKPyV seroreactivity groups measured before transplantation. Association between baseline donor and recipient BKPyV seroreactivity groups and the combination of both with onset of posttransplantation recipient BKPyV viremia was determined by Cox regression. Uni- and multivariate Cox regressions were performed to determine which additional baseline covariates affected development of BKPyV viremia and BKPyVAN. The chi-square test, Fisher exact test or Mann-Whitney *U* test was used for evaluating differences of BKPyV viremia characteristics between viremic recipients with and without BKPyVAN. For all performed tests, a p-value <0.05 in a two-sided test was considered statistically significant.

Results

In total, 111 of 407 recipients (27%) became viremic during follow-up (Table 1, Figure S1), the majority within

Table 1: Donor, recipient, and transplantation characteristics sorted for BKPyV viremia and BKPyVAN among 407 kidney transplantation recipients in the first year after kidney transplantation

	All recipients (n = 407)			Viremic recipients (n = 111)		
	No BKPyV viremia (n = 296)	BKPyV viremia (n = 111)	p-value[1]	No BKPyVAN (n = 99)	BKPyVAN (n = 12)	p-value[1]
Donor						
Age (years)	53 (11.7)	54 (11.5)	0.354	54 (11.7)	57 (9.6)	0.386
Sex						
Male	119 (40%)	42 (38%)	0.664	37 (37%)	5 (42%)	0.763
Recipient						
Age (years)	50 (13.5)	53 (14.2)	0.080	53 (14.1)	53 (16.1)	0.790
Sex						
Male	177 (60%)	73 (66%)	0.271	65 (66%)	8 (67%)	1.000
Underlying condition[2]						
Inherited	72 (24%)	26 (23%)	0.239	22 (22%)	4 (33%)	0.411
Glomerular	80 (27%)	26 (23%)		23 (23%)	3 (25%)	
Vascular	55 (19%)	32 (29%)		31 (31%)	1 (8%)	
Obstructive	27 (9%)	7 (6%)		6 (6%)	1 (8%)	
Other	62 (21%)	20 (18%)		17 (17%)	3 (25%)	
Dialysis pretransplantation	182 (62%)	64 (58%)	0.482	57 (58%)	7 (58%)	1.000
Duration dialysis (mo)	12 (18.4)	9 (12.1)	0.106	9 (11.6)	12 (15.9)	0.730
PRA pretransplantation						
Nonimmunized[3]	284 (96%)	108 (97%)	0.768	97 (98%)	11 (92%)	0.293
Monoclonal antibody						
Basiliximab	277 (94%)	103 (93%)	0.776	92 (93%)	11 (92%)	1.000
Alemtuzumab	19 (6%)	8 (7%)		7 (7%)	1 (8%)	
Calcineurin inhibitor						
Cyclosporin A	70 (24%)	27 (24%)	0.887	27 (27%)	0 (0%)	0.037
Tacrolimus	226 (76%)	84 (76%)		72 (73%)	12 (100%)	
Proliferation inhibitor						
Azathioprine	0 (0%)	1 (<1%)	0.273	1 (1%)		1.000
Everolimus	1 (<1%)	0 (0%)	1.000	0 (0%)	0 (0|%)	n.p.
Mycophenolate mofetil	295 (100%)	110 (99%)	0.472	98 (99%)	0 (0%)	1.000
Corticosteroids	296 (100%)	111 (100%)	n.p.	99 (100%)	12 (100%)	n.p.
Rejection treatment[4]	61 (21%)	31 (28%)	0.116	22 (22%)	9 (75%)	<0.001
Transplantation						
Retransplantation	25 (8%)	11 (10%)	0.650	9 (9%)	2 (17%)	0.339
Year of transplantation						
Before 2007	43 (15%)	18 (16%)	0.671	18 (18%)	0 (0%)	0.209
2007 to present	253 (85%)	93 (84%)		81 (82%)	12 (100%)	
Unrelated donor	144 (49%)	67 (60%)	0.035	58 (59%)	9 (75%)	0.357
Blood group						
Compatible[5]	283 (96%)	104 (94%)	0.341	92 (93%)	12 (100%)	1.000
HLA mismatched						
A, B and DR loci[6]						
0	17 (6%)	6 (5%)	0.888	6 (6%)	0 (0%)	0.437
1–3	143 (48%)	51 (46%)		47 (48%)	4 (33%)	
4–6	136 (46%)	54 (49%)		46 (46%)	8 (67%)	

Data are shown as mean (standard deviation) or n (%).

BKPyV, BK polyomavirus; BKPyVAN, BK polyomavirus-associated nephropathy; n.p., not possible; PRA, panel reactive antibodies.

[1]The p-values were calculated using the chi-square test, Fisher exact test, or Student t-test. A p-value <0.05 was considered statistically significant.

[2]Inherited diseases include autosomal dominant polycystic kidney disease, medullary cystic disease, cystic kidney disease not otherwise specified, arteriovenous malformation due to Klippel–Trénaunay–Weber syndrome, familiar erythrocyturia, Alport syndrome, familiar focal segmental glomerulosclerosis by NPHS2 mutation, familiar hemolytic uremic syndrome, and kidney dys- and agenesis; Glomerular diseases include membranous nephropathy, IgA nephropathy, systemic lupus erythematosus, proliferative glomerulonephritis, membranoproliferative glomerulonephritis, focal segmental glomerulosclerosis, pauci-immune crescentic glomerulonephritis, Morbus Wegener, ANCA-associated vasculitis, anti–glomerular basement membrane nephritis, global glomerulosclerosis, and immunotactoid glomerulonephritis. Vascular diseases include diabetes mellitus types I and II, hypertension, nephrosclerosis, hemolytic uremic syndrome, arteria renalis stenosis and thrombotic microangiopathy. Obstructive diseases include reflux nephropathy, urethral valves, nephrolithiasis, obstructive uropathy and prostate hypertrophy. Other diseases include chronic pyelonephritis, acute tubular necrosis, tubulointerstitial nephritis, lithium nephropathy, urate and analgesic nephropathy, iatrogenic disease and unknown underlying condition.

[3]PRA immunization: nonimmunized, PRA 0–5%; immunized, PRA 6–99%.

[4]Rejection treatment consisted of methylprednisolone 1000 mg intravenously once daily for 3 days.

[5]Blood group data of one donor–recipient pair is missing; the recipient was BKPyV viremia negative.

[6]HLA mismatched (A, B and DR loci) arranged in groups with no mismatches (completely matched), one to three mismatches (haplotype mismatched), and four or more mismatches (more than haplotype mismatch).

6 mo after transplantation (Figure 1), and 87 of them (79%) with sustained viremia (Figure S1). The median peak viral load was 6.9×10^3 c/mL (interquartile range 8.8×10^2–4.2×10^5 c/mL). Peak viral loads $>10^4$ c/mL were particularly prevalent among recipients that developed viremia within the first 6 mo after transplantation (Figure 1).

BKPyVAN was diagnosed in only 12 participants (3%) (Table 1, Figure S1), probably because tapering of immunosuppression was implemented on detection of viremia. All recipients diagnosed with BKPyVAN had peak BKPyV loads $\geq 10^4$ c/mL (Table S1), and both peak BKPyV load and area under the curve of BKPyV load during follow-up were significantly associated with development of BKPyVAN ($p < 0.001$) (Table S1).

The incidence of BKPyV viremia and BKPyVAN during follow-up was compared with specific donor, recipient, and transplantation characteristics (Table 1). No significant differences were observed between viremic and nonviremic recipients with regard to any of the listed donor or recipient baseline characteristics, including underlying condition, immunosuppressive regime and PRA immunization. With respect to type of transplantation, BKPyV viremia was more common among recipients from unrelated donors (60% vs. 49%, $p = 0.035$). Blood group compatibility and HLA matching were not significantly different between viremic and nonviremic recipients. As anticipated, use of tacrolimus (Table 1) and rejection treatment with prednisolone (Tables 1 and 5) were associated with development of BKPyVAN in our cohort.

To investigate the association between BKPyV seroreactivity and, BKPyV viremia or BKPyVAN during follow-up, baseline BKPyV VP1 IgG seroresponses were measured in both donors and recipients (Figure S2). In total, 389 (96%) of the donors and 385 (95%) of the recipients were BKPyV seropositive (Table 2). In line with the high seroprevalence in both groups, neither BKPyV serostatus nor specific donor–recipient serostatus combinations were associated with BKPyV viremia and BKPyVAN (Table 2). Nevertheless, when the level of donor and recipient BKPyV IgG seroresponses were analyzed, either as a continuous variable or categorized in quartiles (Figure S2), statistically significant associations were observed between pretransplantation donor seroreactivity

Table 2: Pretransplantation BKPyV seropositivity and seroreactivity among kidney allograft donors and recipients, related to posttransplantation recipient BKPyV viremia and BKPyVAN

	Recipients (n = 407)			Viremic recipients (n = 111)		
	No BKPyV viremia (n = 296)	BKPyV viremia (n = 111)	p-value[1]	No BKPyVAN (n = 99)	BKPyVAN (n = 12)	p-value[1]
Donor						
BKPyV seropositive	281 (95%)	108 (97%)	0.420	377 (95%)	12 (100%)	0.670
BKPyV seroreactivity	11 511 (7371)	17 200 (6605)	<0.001	12 883 (7609)	18 988 (4199)	<0.001
Seroreactivity quartile groups[2]						
Low (Q1)	93 (31%)	9 (8%)	<0.001	102 (26%)	0 (0%)	0.013
Low intermediate (Q2)	82 (28%)	21 (19%)		102 (26%)	1 (8%)	
High intermediate (Q3)	71 (24%)	30 (27%)		95 (24%)	6 (50%)	
High (Q4)	50 (17%)	51 (46%)		96 (24%)	5 (42%)	
Recipient						
BKPyV seropositive	283 (96%)	102 (92%)	0.140	374 (95%)	11 (92%)	1.000
BKPyV seroreactivity	13 774 (7834)	12 342 (7956)	0.103	13 422 (7901)	12 119 (7492)	0.573
Seroreactivity quartile groups[2]						
Low (Q1)	68 (23%)	34 (31%)	0.219	98 (25%)	4 (33%)	0.977
Low intermediate (Q2)	76 (26%)	26 (23%)		99 (25%)	3 (25%)	
High intermediate (Q3)	72 (24%)	30 (27%)		99 (25%)	3 (25%)	
High (Q4)	80 (27%)	21 (19%)		99 (25%)	2 (17%)	
Donor–recipient pair						
BKPyV serostatus						
+/+	268 (91%)	100 (90%)	0.107	357 (90%)	11 (92%)	0.707
+/−	13 (4%)	8 (7%)		20 (5%)	1 (8%)	
−/+	15 (5%)	2 (2%)		17 (4%)	0 (0%)	
−/−	0 (0%)	1 (<1%)		1 (<1%)	0 (0%)	

Data are shown as mean (standard deviation) or n (%).
+, BKPyV seropositive; −, BKPyV seronegative; BKPyV, BK polyomavirus; BKPyVAN, BK polyomavirus-associated nephropathy; Q, quartile.
[1]The p-values were calculated using the chi-square test, Fisher exact test, or Student t-test. A p-value <0.05 was considered statistically significant.
[2]Mean fluorescence intensity distributions of the donor and recipient seroreactivity quartile groups can be found in the legend of Figure S2.

and posttransplantation recipient BKPyV viremia ($p < 0.001$ and $p < 0.001$, respectively) (Table 2) and BKPyVAN ($p < 0.001$ and $p = 0.013$, respectively) (Table 2). To illustrate, only nine (8%) of the viremic recipients had a low seroreactive donor (quartile 1 [Q1]), whereas 51 (46%) had a high seroreactive donor (Q4) (Table 2). The same statistically significant trend was observed for BKPyVAN; just one instance (8%) occurred in recipients with an (intermediate) low seroreactive donor (Q1–Q2), whereas the majority (11 of 12, 92%) developed in recipients with an (intermediate) high seroreactive donor (Q3–Q4) (Table 2). In contrast, pretransplantation BKPyV seroreactivity of the recipient was not associated with viremia or BKPyVAN (Table 2). To confirm the associations observed for BKPyV seroreactivity, donor BKPyV IgG levels were reassessed with ELISA by a different laboratory that generated comparable results (Figures S3A and B).

To further substantiate the observed association between pretransplantation donor BKPyV IgG levels and posttransplantation recipient viremia, Kaplan–Meier curves were generated to compare the onset of BKPyV viremia stratified for baseline BKPyV seroreactivity quartiles of donors and recipients. Again, a strong and highly significant correlation was observed between recipient viremia and donor BKPyV seroreactivity ($p < 0.001$) (Figure 2). The Kaplan–Meier curves based on the donor BKPyV seroreactivity results of the conformational ELISA showed the same effect ($p < 0.001$) (Figure 2, inset). For recipient BKPyV seroreactivity, a nonsignificant reverse trend was found (Figure S4A).

To estimate the risk indicated by baseline BKPyV seroreactivity, the hazard ratio (HR) for recipient viremia was calculated. With every 5000 MFI unit increase of donor seroreactivity, the HR increased by 1.59 (95% confidence interval [CI] 1.38–1.84, $p < 0.001$) (Table 3). In recipients from high BKPyV-seroreactive donors, the HR was 6.92 (95% CI 3.41–14.06, $p < 0.001$) (Table 3). In highly seroreactive recipients, the risk of viremia tended to decrease (HR 0.57, 95% CI 0.33–0.98, $p = 0.041$) (Table 3).

Because opposite trends were observed for donor and recipient baseline BKPyV seroreactivity, the interplay between these potentially predictive factors of posttransplantation BKPyV viremia was analyzed by calculating the BKPyV viremia risk for donor BKPyV seroreactivity stratified by recipient seroreactivity. As shown in Figure S4(B), a combination of these factors resulted in a substantially increased risk of BKPyV viremia in low-BKPyV–seroreactive recipients receiving an allograft from a highly seroreactive donor (HR 10.07, 95% CI 3.50–28.96, $p < 0.001$) (Table 3).

Finally, Cox regression analyses were performed for the risk of developing BKPyV viremia after transplantation related to the identified serological risk factors

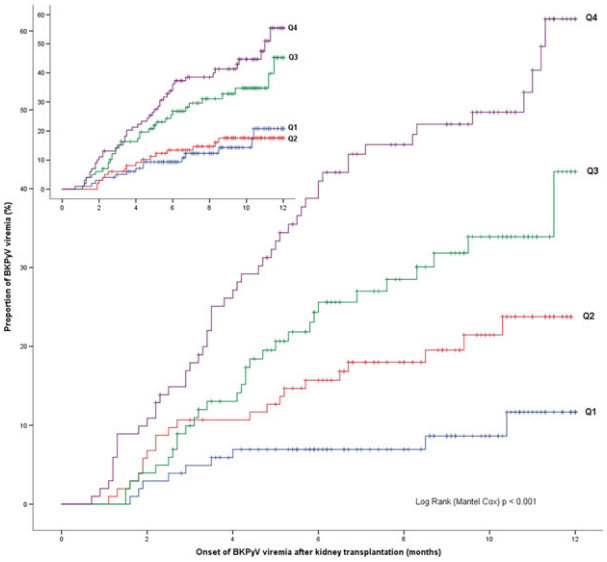

Figure 2: Proportion of BKPyV viremia detected in the first year after kidney transplantation according to BKPyV seroreactivity determined in donors. Kaplan–Meier (1 − survival function) curves for proportion of BKPyV viremia observed in the recipients according to donor BKPyV seroreactivity quartile groups (shown in Figure S2). The inset shows the Kaplan–Meier (1 − survival function) curves for proportion of BKPyV viremia observed in the recipients according to donor BKPyV seroreactivity quartile groups determined with enzyme-linked immunosorbent assay (log rank [Mantel Cox] $p < 0.001$). Mean fluorescence intensity distributions of the donor seroreactivity quartile groups are described in the legend of Figure S2. Tick marks represent censored recipients. Groups from the inset are divided by optical density values for seroreactivity quartiles. Q1, low: 0–0.0150; Q2, low intermediate: 0.151–0.6105; Q3, high intermediate: 0.6106–1.2225; Q4, high: 1.2226–3.1180. Tick marks represent censored recipients. BKPyV, BK polyomavirus; Q, quartile.

and the cohort characteristics presented in Table 1. In the univariate analysis (Table 4), apart from donor BKPyV seroreactivity (HR 1.59, 95% CI 1.38–1.84, $p < 0.001$), only unrelatedness of the living donor (HR 1.49, 95% CI 1.02–2.17, $p = 0.042$) and rejection treatment (HR 1.54, 95% CI 1.02–2.34, $p = 0.040$) were associated with BKPyV viremia. Recipient BKPyV seroreactivity did not reach statistical significance (HR 0.90, 95% CI 0.80–1.02, $p = 0.088$). In line with the stratified analysis described earlier, the multivariate analysis showed a significant protective effect of recipient BKPyV seroreactivity (HR 0.84, 95% CI 0.75–0.95, $p = 0.006$) (Table 4). The effects of the unrelated donor (HR 1.35, 95% CI 0.79–2.31, $p = 0.268$) and rejection treatment (HR 1.53, 95% CI 0.97–2.40, $p = 0.066$) were lost in the multivariate analysis, whereas donor BKPyV seroreactivity remained a highly significant risk factor for BKPyV viremia (HR 1.61, 95% CI 1.39–1.88, $p < 0.001$).

Despite the low number of BKPyVAN cases (n = 12), univariate analysis for BKPyVAN (Table 5) showed an association with donor BKPyV IgG levels (HR 1.95, 95% CI 1.15–3.32, p = 0.013) and rejection treatment (HR 11.60, 95% CI 3.14–42.86, p < 0.001). These associations were also observed in the multivariate analysis (HR 2.89, 95% CI 1.33–6.29, p = 0.007, and HR 23.52, 95% CI 4.57–120.99, p < 0.001, respectively). Recipient seroreactivity showed a reverse but not statistically significant protective trend in the univariate and multivariate analyses (HR 0.91, 95% CI 0.64–1.30, p = 0.609, and HR 0.69, 95% CI 0.46–1.04, p = 0.075, respectively). In a subanalysis for BKPyVAN among viremic recipients, in which recipients of donors with high IgG levels are overrepresented, no additional associations were found in either uni- or multivariate analysis (Table S2).

Discussion

BKPyV-associated disease is a major problem in the care of kidney transplant recipients for whom no antiviral treatment is available (1,2,10,32). Because timely reduction of immunosuppression is the only effective treatment to date (11,12), all recipients are currently screened for BKPyV viremia on a regular basis after transplantation (10–12,16). Only a subset of recipients, however, is at risk of developing BKPyV viremia (15–30%) and, subsequently, BKPyVAN (1–10%) (10,12–16). Apart from rejection treatment following transplantation, pretransplantation risk factors for BKPyV viremia and BKPyVAN have not been identified; therefore, no markers are available to predict which recipients are actually at risk. The observed strong positive correlation between donor BKPyV IgG levels and development of BKPyV viremia and BKPyVAN in recipients could fill this gap.

The kidney allograft plays a key role in the development of BKPyVAN either because the allograft serves as a transmitting vehicle for BKPyV to the recipient, as suggested by a number of previous reports (19,23), or because of increased renal vulnerability to infection, for example, resulting from kidney injury related to transplantation (10,33). Our findings that show strong associations between donor BKPyV seroreactivity and subsequent BKPyV viremia and BKPyVAN provide strong support for the first explanation, indicating that

Table 3: Risk of recipient BKPyV viremia after kidney transplantation according to BKPyV seroreactivity measured before transplantation in the donor, in the recipients, and in the donor–recipient pairs by stratified analysis

		HR	95% CI	p-value[1]
Donor				
BKPyV seroreactivity[2]		1.59	1.38–1.84	<0.001
Seroreactivity quartile groups[3]				
Low (Q1)		1.0		<0.001
Low intermediate (Q2)		2.34	1.07–5.11	0.033
High intermediate (Q3)		3.82	1.82–8.06	< 0.001
High (Q4)		6.92	3.41–14.06	< 0.001
Recipient				
BKPyV seroreactivity[2]		0.90	0.80–1.02	0.088
Seroreactivity quartile groups[3]				
Low (Q1)		1.0		0.221
Low intermediate (Q2)		0.74	0.47–1.24	0.257
High intermediate (Q3)		0.85	0.52–1.39	0.509
High (Q4)		0.57	0.33–0.98	0.041
Donor–recipient pair				
Donor seroreactivity[3]	Recipient seroreactivity[3]			
Low (Q1)	High (Q3–Q4)	1.00		<0.001
	Low (Q1–Q2)	0.90	0.24–3.36	0.879
Intermediate low (Q2)	High (Q3–Q4)	1.84	0.55–6.12	0.319
	Low (Q1–Q2)	2.52	0.82–7.72	0.106
Intermediate high (Q3)	High (Q3–Q4)	2.99	0.97–9.16	0.056
	Low (Q1–Q2)	4.31	1.45–12.82	0.009
High (Q4)	High (Q3–Q4)	4.89	1.71–14.01	0.003
	Low (Q1–Q2)	10.07	3.50–28.96	<0.001

BKPyV, BK polyomavirus; CI, confidence interval; HR, hazard ratio; MFI, mean fluorescence intensity; Q, quartile.
[1]The p-values, HRs and 95% CIs were calculated with Cox regression analysis. A p-value <0.05 was considered statistically significant.
[2]Donor and recipient seroreactivity per 5000 increasing MFI.
[3]MFI distributions of the donor and recipient seroreactivity quartile groups can be found in the legend of Figure S2.

Table 4: Uni- and multivariate Cox regression analysis for risk factors of BK polyomavirus viremia development among 407 kidney transplantation recipients in the first year after transplantation

	Univariate analysis			Multivariate analysis		
Covariate	HR	95% CI	p-value[1]	HR	95% CI	p-value[1]
Age, recipient (years)	1.01	1.00–1.03	0.138	1.00	0.99–1.02	0.665
Age, donor (years)	1.01	0.99–1.02	0.498	1.00	0.98–1.02	0.858
Sex, recipient	1.21	0.82–1.80	0.340	1.04	0.68–1.60	0.842
Sex, donor	0.90	0.62–1.33	0.603	0.94	0.61–1.44	0.765
Underlying condition[2]						
Inherited	1.00		0.359	1.00		0.476
Glomerular	0.97	0.56–1.66	0.903	1.14	0.64–2.04	0.657
Vascular	1.45	0.86–2.43	0.163	1.62	0.94–2.77	0.081
Obstructive	0.78	0.34–1.79	0.554	1.04	0.43–2.51	0.939
Other	0.94	0.52–1.67	0.820	1.18	0.64–2.17	0.601
Dialysis pretransplantation	0.85	0.59–1.24	0.405	0.89	0.55–1.41	0.608
Duration dialysis (mo)	0.90	0.77–1.04	0.156	0.99	0.98–1.01	0.219
Unrelated donor	1.49	1.02–2.17	0.042	1.35	0.79–2.31	0.268
Retransplantation	1.23	0.66–2.30	0.513	1.36	0.68–2.68	0.384
PRA immunization pretransplantation	0.72	0.23–2.26	0.570	0.72	0.22–2.41	0.598
Blood group compatibility	1.43	0.67–3.08	0.359	1.22	0.44–3.42	0.701
HLA mismatched A, B and DR loci[3]						
0	1.00		0.832	1.00		0.746
1–3	1.08	0.46–2.52	0.859	0.82	0.34–2.01	0.668
4–6	1.20	0.51–2.78	0.678	0.71	0.27–1.89	0.495
Basiliximab versus alemtuzumab	1.04	0.51–2.13	0.921	0.99	0.38–2.58	0.988
Tacrolimus versus cyclosporin A	0.89	0.58–1.37	0.599	0.76	0.48–1.23	0.264
Donor BKPyV seroreactivity[4]	1.59	1.38–1.84	<0.001	1.61	1.39–1.88	< 0.001
Recipient BKPyV seroreactivity[4]	0.90	0.80–1.02	0.088	0.84	0.75–0.95	0.006
Rejection treatment [5]	1.54	1.02–2.34	0.040	1.53	0.97–2.40	0.066

CI, confidence interval; HR, hazard ratio; PRA, panel reactive antibodies.
[1]The p-values, HRs and 95% CIs were calculated with uni- and multivariate Cox regression analyses. A p-value <0.05 was considered statistically significant.
[2]Describes the HR of viremia in recipients with each underlying condition by group compared with the inherited disease group; clarification of the categories can be found in the legend of Table 1.
[3]Describes the onset of viremia in recipients with each group of number of HLA mismatches on loci A, B and DR compared with the group with no HLA mismatches.
[4]Donor and recipient seroreactivity per 5000 increasing mean fluorescence intensity.
[5]Rejection treatment consisted of methylprednisolone 1000 mg intravenously once daily for 3 days.

manifest BKPyV infection in recipients originates from the kidney allograft.

The strength of the observed association between donor BKPyV seroreactivity and recipient BKPyV infection and the high calculated hazard levels are remarkable. As far as we know, previous studies have not compared donor BKPyV seroreactivity with recipient viremia and BKPyVAN. In general, studies that compared recipient BKPyV infection with BKPyV-related virological and immunological characteristics are rare, probably because BKPyV serology was considered not useful in this regard; BKPyV serostatus was shown to be positive in almost all cases for donors as well as recipients. One study compared donor BKPyV IgG levels and recipient BKPyV viruria and noted a correlation, in line with our findings (19). Unfortunately, urine samples could not be analyzed in the present cohort because they were not routinely archived. Despite this and some other limitations of the study, discussed below, our findings indicate that BKPyV seroreactivity is the strongest (donor-related) pretransplant factor identified to date, predicting manifest BKPyV infection in kidney allograft recipients.

The predictive value of high donor BKPyV IgG levels for recipient BKPyV infection makes one wonder about the role of humoral BKPyV immunity in BKPyV infection. It is not donor-derived BKPyV-directed antibodies that confer infection but rather the virus itself. Consequently, we assume that the intensity of measured donor BKPyV seroreactivity reflects the amount or virulence of infectious BKPyV present in the persistently infected kidney allograft. Because we are unaware of documented differences in virulence among BKPyV genotypes, it is most likely that BKPyV IgG levels reflect the BKPyV kidney load, as such, correlating with the risk of BKPyV infection in recipients. It should be noted, however, that BKPyV

Table 5: Uni- and multivariate Cox regression analysis for risk of BKPyVAN development among recipients (n = 407) in the first year after kidney transplantation

	Univariate analysis			Multivariate analysis		
Covariate	HR	95% CI	p-value[1]	HR	95% CI	p-value[1]
Age, recipient (years)	1.01	0.97–1.05	0.638	0.98	0.92–1.03	0.424
Age, donor (years)	1.03	0.98–1.08	0.284	1.06	0.98–1.14	0.136
Sex, recipient	1.26	0.38–4.20	0.702	1.23	0.29–4.38	0.864
Sex, donor	1.08	0.34–3.41	0.893	0.82	0.21–3.25	0.779
Underlying condition[2]						
Inherited	1.00		0.838	1.00		0.350
Glomerular	0.68	0.15–3.05	0.618	0.49	0.08–2.83	0.422
Vascular	0.28	0.03–2.46	0.248	0.25	0.02–2.54	0.240
Obstructive	0.73	0.08–6.56	0.781	3.03	0.23–40.27	0.402
Other	0.88	0.20–3.94	0.868	2.60	0.42–15.91	0.302
Dialysis pretransplantation	0.92	0.29–2.90	0.883	0.35	0.07–1.75	0.198
Duration dialysis (mo)	1.03	0.70–1.50	0.894	1.01	0.97–1.05	0.657
Unrelated donor	2.90	0.79–10.71	0.110	1.58	0.30–8.33	0.593
Retransplantation	2.06	0.45–9.41	0.350	2.94	0.36–24.14	0.316
PRA immunization pretransplantation	2.58	0.33–20.00	0.364	3.05	0.22–42.12	0.405
Basiliximab versus alemtuzumab	1.21	0.16–9.35	0.857	3.59	0.35–37.16	0.283
Donor BKPyV seroreactivity[4]	1.95	1.15–3.32	0.013	2.89	1.33–6.29	0.007
Recipient BKPyV seroreactivity[4]	0.91	0.64–1.30	0.609	0.69	0.46–1.04	0.075
Rejection treatment[5]	11.60	3.14–42.86	<0.001	23.52	4.57–120.99	<0.001

BKPyV, BK polyomavirus; BKPyVAN, BK polyomavirus-associated nephropathy; CI, confidence interval; HR, hazard ratio; PRA, panel reactive antibodies.

[1]The covariates blood group compatibility, HLA mismatches and tacrolimus versus cyclosporin A, as shown in Table 1, could not be added to this Cox model because of the low number of BKPyVAN cases in our cohort and the distribution of these baseline characteristics among the recipient groups with and without BKPyVAN (Table 1).

[2]The p-values, HRs and 95% CIs were calculated with uni- and multivariate Cox regression analyses. A p-value <0.05 was considered statistically significant.

[3]Describes the HR of BKPyVAN in recipients with each underlying condition group compared with the inherited disease group; clarification of the categories can be found in the legend of Table 1.

[4]Donor and recipient seroreactivity per 5000 increasing mean fluorescence intensity.

[5]Rejection treatment consisted of methylprednisolone 1000 mg intravenously once daily for 3 days.

genotyping is currently skewed toward virus isolates obtained from recipients with BKPyV infection and thus may not represent the distribution of BKPyV genotypes circulating in the general population including potential donors.

Because BKPyV seroreactivity likely reflects the BKPyV load of infected kidneys, it is important to consider the role that serum IgG antibodies play in the control of persistent BKPyV infection. Because previous studies have shown that BKPyV IgG seroresponses increase on BKPyV DNA detection (21,24,25), and in line with observations suggesting an inverse relationship between recipient BKPyV IgG levels and BKPyV infection (19,21,22), BKPyV-directed antibodies might be directly involved in containment of BKPyV infection. Recent studies by Randhawa and Buck have provided evidence of efficient BKPyV neutralization by BKPyV-directed serum antibodies (34,35), and proposed the possibility of offering recipients intravenous immunoglobulins in the posttransplantation period. Involvement of BKPyV-specific antibodies in controlling BKPyV infection can also be inferred from the increased risk of BKPyV viremia observed in seronegative recipients. Alternatively, especially in the recipients, the measured BKPyV seroresponses may be a marker of another relevant component of the immune system, for example, BKPyV-specific T cells that are essential in controlling BKPyV infection after transplantation (21,36). This possibility is underscored by a recent study by Schmidt et al that reports a strong correlation in recipients with BKPyV replication between BKPyV IgG levels and the percentage of BKPyV-reactive CD4 T cells (37).

Taken together, it is important to realize that in the context of transplantation and prediction of BKPyVAN, BKPyV seroreactivity might actually reflect both the BKPyV kidney load and BKPyV T cell immunity. In donors, as depicted earlier, BKPyV seroreactivity likely reflects BKPyV graft load. In recipients, however, BKPyV seroreactivity might primarily be regarded as a reflection of the overall immunity against BKPyV, including T cells. Both high donor BKPyV-specific antibody titers and low (or absent) recipient BKPyV-specific antibody titers are mentioned as risk factors for BKPyVAN in the most recent American Society of Transplantation Infectious

Disease Community of Practice guideline (38). The added value of our study lies particularly in the integrated evaluation of this serological marker among donor–recipient pairs because this provides leads for future algorithms to predict BKPyV-related disease after transplantation.

Possible limitations of our study include the single-center design and the fact that not all recipients were sampled at every time point after transplantation; however, we are not aware of geographic variability regarding BKPyV seroreactivity and have no indication that completion of the sample set would have changed the overall conclusions. The fact that not all recipients with BKPyV viremia $\geq 10^4$ c/mL were biopsy screened could have caused underrecognition of the number of BKPyVAN cases; therefore, statistics performed to calculate BKPyVAN risk must be interpreted with caution. Nevertheless, even with this small number, statistically significant results were obtained regarding the association between recipient BKPyVAN and donor BKPyV seroreactivity. Because of the inclusion of only living donor–recipient pairs, it remains uncertain whether these results also apply to deceased donor–recipient pairs. A borderline increased risk of BKPyV viremia was observed in recipients that received a kidney allograft from an unrelated donor compared with recipients from related donors; however, it is not expected that this factor influenced the observed associations between donor BKPyV seroreactivity and recipient BKPyV viremia and BKPyVAN. In general, both the incidence and load of viremic episodes observed in the present study population are in line with comparable kidney transplantation cohorts reported in the literature, including cohorts with deceased donors (10,14,16,25).

Despite its possible limitations, this study identified a serological marker that indicates the risk of BKPyV infection after kidney transplantation. The results suggest that a single pretransplantation BKPyV IgG measurement could be used to assess the risk of BKPyV infection after transplantation. Because our data show that recipient BKPyV seroreactivity modulates the risk determined by donor BKPyV seroreactivity, it appears most useful to determine BKPyV seroreactivity before transplantation in both the allograft donor and recipient. Subsequent studies are needed to reveal whether a pretransplantation serological BKPyV risk assessment could provide a basis for personalized BKPyV load–monitoring strategies aimed at early identification of BKPyV viremic patients to increase the efficiency of BKPyV screening. Furthermore, it might be worthwhile to consider the additive value of donor–recipient BKPyV seroreactivity matching (a highly seroreactive donor calls for a highly seroreactive recipient) to lower the BKPyV infection risk. Passive immunization of recipients at high risk could also be considered an option based on the protective effect of high IgG levels in recipients. The relevance of the present findings for other (reactivating) viral infections after solid organ transplantation merits further study.

By studying BKPyV seroresponses, a strong correlation was identified between baseline BKPyV IgG levels and posttransplantation BKPyV infection. Use of BKPyV seroreactivity as a practical predictive disease marker could be of great value in the management of BKPyV-associated disease. Moreover, these findings call for further study into approaches aimed at improving humoral BKPyV immunity after transplantation, such as the administration of (BKPyV-specific) intravenous immunoglobulins and BKPyV vaccination.

Acknowledgments

This study was supported by the Dutch Kidney Foundation, grant 13A1D302.

Disclosure

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

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Table S1: Characteristics of BK polyomavirus viremia among recipients who did or did not develop BK polyomavirus-associated nephropathy.

Table S2: Uni- and multivariate Cox regression analyses for risk of development of BK polyomavirus-associated nephropathy among BK polyomavirus viremic recipients (n = 111) during the first year after kidney transplantation.

Figure S1: Study population, inclusion of kidney transplantation donor–recipient pairs, and development of BKPyV viremia and BKPyVAN divided by donor and recipient pretransplantation BKPyV serostatus. Inclusion and exclusion criteria and distribution of BKPyV viremia (p = 0.107), sustained BKPyV viremia (p = 0.107), BKPyV viremia of log ≥4 (p = 0.155), and BKPyVAN (p = 0.707) in the different BKPyV-serostatus pretransplantation donor–recipient pair combinations. The p-values were calculated using the Fisher exact test. A p-value <0.05 was considered statistically significant. +, BKPyV seropositive; −, BKPyV seronegative; BKPyV, BK polyomavirus; BKPyVAN, BK polyomavirus-associated nephropathy; c/mL, copies per milliliter; D, donor; pre-KTx, pre–kidney transplant; R, recipient.

Figure S2: Pretransplantation IgG seroreactivity of 407 kidney transplantation donors and recipients against the BK polyomavirus (BKPyV) viral capsid protein 1 (VP1) antigen. Pretransplantation IgG seroreactivity of 407 kidney transplantation donors and recipients against the BKPyV VP1 antigen. Each dot represents the pretransplantation BKPyV VP1 IgG seroreactivity of individual donors (left) and recipients (right), tested by a Luminex assay. The measured BKPyV VP1 IgG seroreactivity of donors and recipients is categorized in quartile groups according to the measured mean fluorescence intensity (MFI) values, with quartile 1 (Q1) containing the lowest seroreactive participants and Q4 the highest. The black lines represent the borders between the quartile groups, and the dashed line represents the cutoff value that was used to calculate the percentage of BKPyV seropositivity. MFI ranges for donor seroreactivity quartiles: Q1, low: −1001 to 6169; Q2 low intermediate: 6170–13 842; Q3 high intermediate: 13 843–20 251; Q4 high: 20 252–24 120. MFI ranges for recipient seroreactivity quartiles: Q1, low: −510 to 6178; Q2, low intermediate: 6179–13 490; Q3, high intermediate: 13 491–21 043; Q4, high: 21 043–24 207.

Figure S3: Baseline donor BKPyV seroreactivity comparison between data generated with the Luminex BKPyV GST–VP1 fusion protein assay and the BKPyV VP1 VLP ELISA. (A) Overall, 396 of 407 (97.3%) pretransplantation donor sera were analyzed by both the Luminex BKPyV GST-VP1 fusion protein assay and the BKPyV VP1 VLP ELISA. The correlation is shown between the MFI values determined by Luminex and the OD values determined by ELISA. (B) The Spearman correlation coefficient was calculated between the MFI and OD values obtained with Luminex and ELISA. A strong positive monotonic correlation was observed between the two variables: $r = 0.823$, $n = 396$, $p < 0.001$. BKPyV, BK polyomavirus; ELISA, enzyme-linked immunosorbent assay; GST, glutathione S-transferase; MFI, mean fluorescence intensity; OD, optical density; VLP, viruslike particle; VP1, viral capsid protein 1.

Figure S4: Proportion of BKPyV viremia detected in the first year after kidney transplantation according to BKPyV seroreactivity determined in recipients and in donor–recipient pairs. (A) Kaplan–Meier (1 − survival function) curves for proportion of BKPyV viremia observed in the recipients according to recipient BKPyV seroreactivity quartile groups (shown in Figure S2). Mean fluorescence intensity distributions of the recipient seroreactivity quartile groups can be found in the legend of Figure S2. Tick marks represent censored recipients. (B) Incidence of recipient BKPyV viremia during follow-up according to donor BKPyV seroreactivity quartile groups stratified for recipient BKPyV seroreactivity measured before transplantation. The overall percentages of BKPyV viremic recipients are shown within each donor–recipient seroreactivity quartile combination. BKPyV, BK polyomavirus; OD, optical density; Q, quartile.