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ORIGINAL ARTICLE

Different risk factor profiles distinguish early-onset from late-onset BKV-replication

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Summary

Two of three reactivations of latent BKV-infection occur within the first 6 months after renal transplantation. However, a clear differentiation between early-onset and late-onset BKV-replication is lacking. Here, we studied all kidney transplant recipients (KTRs) at our single transplant center between 2004 and 2012. A total of 103 of 862 KTRs were diagnosed with BK viremia (11.9%), among which 24 KTRs (2.8%) showed progression to BKV-associated nephropathy (BKVN). Sixty-seven KTRs with early-onset BKV-replication (65%) and 36 KTRs with late-onset BKV-replication (35%) were identified. A control group of 598 KTRs without BKV-replication was used for comparison. Lymphocyte-depleting induction, CMV-reactivation, and acute rejection increased the risk of early-onset BKV-replication ($P < 0.05$). Presensitized KTRs undergoing renal retransplantation were those at increased risk of late-onset BKV-replication ($P < 0.05$). Among KTRs with BK viremia, higher doses of mycophenolate increased the risk of progression to BKVN ($P = 0.004$). KTRs with progression to BKVN showed inferior allograft function ($P < 0.05$). KTRs with late-onset BK viremia were more likely not to recover to baseline creatinine after BKV-replication ($P = 0.018$). Our data suggest different risk factors in the pathogenesis of early-onset and late-onset BKV-reactivation. While a more intensified immunosuppression is associated with early-onset BKV-replication, a chronic inflammatory state in presensitized KTRs may contribute to late-onset BKV-replication.

Introduction

BK polyomavirus (BKV) was isolated in 1971 from the urine of a kidney transplant recipient (KTR) who suffered from ureteral stenosis and is still – in case of progression to BKV-associated nephropathy (BKVN) – one of the most challenging infectious complications after renal transplantation [1–3]. BKV is known to persist in tubular epithelial cells of kidney, ureter, and bladder with intermittent reactivation and low-level viruria in up to 50% of KTRs [4,5]. Reactivation of BKV from the persistent subclinical state is monitored using quantitative urine and plasma PCR that have been shown to have a high negative predictive value for BKVN [5–7]. In addition to conventional PCR, measurement of BKV-VP1 mRNA in urinary cells and the detection of Haufen in urine have been suggested as noninvasive means to diagnose BKVN [8,9]. Progression to BKVN occurs in 1–10% of KTRs with varying degrees of allograft dysfunction and increasing serum creatinine concentrations over a period of weeks [10].

The gold standard for the diagnosis of BKVN is a tissue biopsy of the allograft kidney, not only to identify BKV inclusions [6,11,12], but also to identify drug toxicity, recurrence of the underlying renal disease, and in particular acute cellular rejection. Very recent work suggested that morphologically resolving BKVN is often characterized by a self-limiting acute interstitial nephritis, that frequently shows negativity for SV-40 staining, and therefore might not be distinguishable from an interstitial cellular rejection [13]. As both BKV-replication and concomitant cellular

rejection may have contributed to allograft damage, the approach to interstitial inflammation by antirejection treatment during and after BKV-replication needs to be discussed on an individual basis.

Potent immunosuppressive drug regimens containing tacrolimus and/or mycophenolate mofetil (MMF) have been suggested to stimulate BKV-replication and to lead to the current high prevalence of BKVN [12,14–16]. BKVN, however, has been reported to occur in a large variety of immunosuppression protocols suggesting that the intensity of the immunosuppression, rather than a specific drug, is a key risk factor. A recent study evaluated the functional activity of BKV-specific T cells *in vitro* in the context of different immunosuppressive drugs and demonstrated that calcineurin inhibitor levels crucially determine the activation of BKV-specific T-cell responses, whereas the contributory immunosuppressive role of MMF, sirolimus, and prednisone had less discernible effect on antigen-specific T-cell activation, but on antigen-specific expansion [17]. A very recent work even suggested a change of risk factors for BK viremia over time with higher overall steroid doses contributing to early BK viremia, and older age and male gender contributing to later BK viremia [18].

Despite a rising number of studies addressing risk factors of BK viremia and BKVN in large cohorts, a clear differentiation between KTRs developing early-onset BK viremia (<6 months after renal transplantation) and KTRs developing late-onset BK viremia (>6 months after renal transplantation) has not been performed. Therefore, we attempted to address following open questions: (i) Are there differences in risk factors between early-onset and late-onset BK viremia? (ii) Are there differences in outcome between early-onset and late-onset BK viremia? (iii) Are there differences in severity and viral kinetics between early-onset and late-onset BK viremia?

Patients and methods

Patients

This study was approved by our local ethical review committee in compliance with the declaration of Helsinki and Istanbul. Informed consent was obtained from all patients.

We examined 862 adult solitary KTRs transplanted at our single transplant center at Charité Campus Virchow Clinic between January 1, 2004 and December 31, 2012 for development of BK viremia. Hundred and three of 862 KTRs (11.9%) were identified with BK viremia among which 24 KTRs (2.8%) showed progression to BKVN and 3 KTRs lost their allograft due to BKVN (12.5%). BKVN diagnosis was based on biopsy with histological findings upon detection of renal dysfunction. BKVN was classified into histological patterns as reported previously [19]. In 16% of KTRs with BK viremia, only kidney biopsies were performed that did not show BKVN.

Kidney transplant recipients were divided into different groups: (i) 24 KTRs with BKVN and 79 KTRs with BK viremia, and (ii) 67 KTRs with early-onset BK viremia (<6 months after renal transplantation) and 36 KTRs with late-onset BK viremia (>6 months after renal transplantation). In addition, the latter group was divided into (i) 51 KTRs with early-onset BK viremia and 16 KTRs with early-onset BKVN, and (ii) 28 KTRs with late-onset BK viremia and 8 KTRs with late-onset BKVN. The definition of early-onset BK viremia as <6 months after transplantation resulted from an almost linearly increasing incidence during the first 6 months post-transplantation and a decline afterward. For analysis of clinical and virological characteristics, KTRs with BK viremia were compared to a control group of 598 KTRs without BK viremia using a multivariate regression analysis adjusted for age, gender, and maintenance immunosuppression.

We compared outcomes of patient survival, death-censored graft survival, and graft function for those who developed BK viremia and those who did not. We excluded recipients with <1 year of follow-up or missing values of serum creatinine in the 6- and 12-month post-transplantation intervals from the analysis. Patients were followed until graft loss, death, or their last patient follow-up date as indicated in the aftercare plan. Estimated glomerular filtration rate (eGFR) was calculated by the abbreviated MDRD equation: $186 \times (\text{creatinine}/88.4) - 1.154 \times (\text{age}) - 0.203 \times (0.742 \text{ if female})$. Detailed clinical and virological characteristics are shown in Tables 1 and 2. To minimize donor variability, a paired kidney analysis was used.

Immunosuppressive therapy

Primary immunosuppression was usually a triple-drug regimen with a calcineurin inhibitor (tacrolimus or cyclosporine), MMF or mycophenolic acid (MPA), and steroid (Table 1). All patients received induction therapy either with an IL-2R antagonist (basiliximab or daclizumab) or with a lymphocyte-depleting agent (OKT 3, antithymocyte globulin, or alemtuzumab).

Recipients of an ABO-incompatible transplant were treated with rituximab, intravenous immunoglobulins, and immunoadsorption therapy, followed by a tacrolimus-based triple-drug immunosuppression. Acute rejections were diagnosed from biopsy histology and graded according to the Banff classification.

Treatment of BKV-associated nephropathy

Therapeutic interventions were applied according to our predefined protocol of switching maintenance immunosuppression and antiviral treatment (Table 2).

Table 1. Patient characteristics.

	BKV group (n = 103)	Control group (n = 598)	P value	BK nephropathy (n = 24)	BK viremia (n = 79)	P value	Early-onset BKV (n = 67)	Late-onset BKV (n = 36)	P value
Age, year	54 (18–76)	53 (18–78)	0.855	55 (24–70)	54 (18–76)	0.815	52 (18–76)	55 (24–76)	0.950
Male sex, n (%)	71 (69)	430 (72)	0.555	17 (71)	54 (68)	0.578	47 (70)	24 (67)	0.824
Cadaveric donation, n (%)	76 (74)	433 (72)	0.812	20 (83)	56 (71)	0.294	47 (70)	29 (81)	0.348
First kidney allograft, n (%)	85 (83)	509 (85)	0.552	20 (83)	65 (82)	1	62 (93)	23 (64)	<0.001
Causes of ESRD, n (%)									
Glomerulonephritis	32 (31)	176 (29)	0.727	7 (29)	25 (32)	1	18 (27)	14 (39)	0.265
Diabetic nephropathy	6 (6)	60 (10)	0.204	2 (8)	2 (3)	0.231	3 (4)	3 (8)	0.419
Nephroangiosclerosis	16 (16)	52 (9)	0.045	4 (17)	14 (18)	1	15 (22)	1 (3)	0.009
Polycystic kidney disease	10 (10)	91 (15)	0.171	3 (13)	7 (9)	0.695	6 (9)	4 (11)	0.737
Uropathy	4 (4)	37 (6)	0.496	0 (0)	4 (5)	0.571	3 (4)	1 (3)	1
Other or undetermined	35 (34)	182 (30)	0.359	8 (33)	27 (34)	1	22 (33)	13 (36)	0.828
Diabetes mellitus, n (%)	19 (18)	123 (21)	0.890	5 (21)	14 (18)	0.767	13 (19)	6 (17)	0.796
Cold ischemia time, hr:min	11:09 (4:00–29:10)	10:40 (2:43–30:20)	0.625	09:15 (4:23–29:10)	11:18 (4:00–22:43)	0.205	10:22 (4:00–29:10)	11:45 (6:55–22:43)	0.756
Time on dialysis, month	59 (0–164)	55 (0–239)	0.362	45 (0–121)	62 (0–164)	0.221	56 (0–158)	64 (0–164)	0.203
CMV seropositivity, n (%)	68 (66)	393 (66)	1	15 (63)	53 (67)	0.806	42 (63)	26 (72)	0.387
CMV viremia, n (%)	57 (55)	200 (33)	<0.001	13 (54)	44 (56)	1	36 (54)	16 (58)	0.412
Concomitant CMV viremia, n (%)	31 (30)	–	–	6 (25)	25 (32)	0.618	28 (42)	3 (8)	<0.001
CMV D+R–, n (%)	15 (15)	93 (16)	0.883	1 (4)	14 (18)	0.183	13 (19)	2 (6)	0.079
BK nephropathy, n (%)	24 (23)	0 (0)	–	24 (100)	0 (0)	–	16 (24)	8 (22)	1
EBV viremia, n (%)	18 (17)	56 (9)	0.021	5 (21)	13 (17)	1	12 (18)	6 (17)	1
Malignancies in follow-up, n (%)	14 (14)	73 (12)	0.746	4 (17)	10 (13)	0.734	10 (15)	4 (11)	0.767
Delayed Graft Function, n (%)	28 (27)	159 (27)	0.904	6 (25)	22 (28)	0.759	19 (28)	9 (25)	0.818
Immunosuppression, n (%)									
Cyclosporine	21 (20)	131 (22)	0.797	2 (8)	19 (24)	0.147	16 (24)	5 (14)	0.308
Tacrolimus	82 (80)	467 (78)	0.797	22 (92)	60 (76)	0.147	51 (76)	31 (86)	0.308
Mycophenolate mofetil	95 (92)	562 (94)	0.509	24 (100)	71 (90)	0.193	63 (94)	32 (89)	0.446
Sirolimus/Everolimus	1 (1)	14 (2)	0.710	0 (0)	1 (1)	–	0 (0)	1 (3)	0.350
Steroids	98 (95)	569 (95)	1	24 (100)	74 (94)	0.588	65 (97)	33 (92)	0.340
Induction with IL-2R antagonist, n (%)	83 (81)	532 (89)	0.022	19 (79)	64 (81)		52 (78)	31 (86)	0.434
Lymphocyte-depleting induction, n (%)	20 (19)	66 (11)	0.022	5 (21)	15 (19)	1	15 (22)	5 (14)	
ABO-incompatible transplants, n (%)	7 (7)	29 (5)	0.465	3 (13)	4 (5)	0.349	4 (6)	3 (8)	0.693
Acute rejection, n (%)									
Total	59 (57)	157 (26)	<0.001	15 (63)	44 (57)	0.641	39 (58)	20 (56)	0.837
Borderline/IA/IB	46 (45)	100 (17)	<0.001	12 (50)	34 (43)	0.641	31 (46)	15 (42)	0.679
IIA/IIB/III	13 (13)	57 (10)	0.372	3 (13)	10 (14)	1	8 (12)	5 (14)	0.765
Concomitant acute rejection, n (%)	36 (35)	–	–	7 (29)	29 (37)	0.627	32 (48)	4 (11)	<0.001
Lymphocyte depletion	6 (6)	50 (8)	0.554	1 (4)	5 (6)	1	5 (7)	1 (3)	0.662

Table 1. continued

	BKV group (n = 103)	Control group (n = 598)	P value	BK nephropathy (n = 24)	BK viremia (n = 79)	P value	Early-onset BKV (n = 67)	Late-onset BKV (n = 36)	P value
2 HLA-A mismatch, n (%)	21 (20)	94 (16)	0.249	4 (17)	17 (22)	0.574	14 (21)	7 (19)	1
2 HLA-B mismatch, n (%)	35 (34)	198 (33)	0.910	10 (42)	25 (32)	0.461	26 (39)	9 (25)	0.194
2 HLA-DR mismatch, n (%)	20 (19)	103 (17)	0.577	2 (8)	18 (23)	0.148	16 (24)	4 (11)	0.190
Total HLA mismatch, n (%)									
4–6 HLA mismatch	42 (41)	210 (35)	0.269	9 (38)	33 (42)	0.814	33 (49)	9 (25)	0.021
PRA, n (%)									
0–10%	95 (92)	554 (93)	0.840	21 (88)	74 (94)	0.385	65 (97)	30 (83)	0.021
>10%	8 (8)	44 (7)		3 (12)	5 (6)		2 (3)	6 (17)	
Donor age, year	53 (13–85)	54 (3–85)	0.580	54 (17–74)	53 (13–85)	0.785	53 (13–85)	48 (19–78)	0.187
Donor male sex, n (%)	43 (42)	301 (50)	0.111	10 (42)	33 (42)	1	29 (43)	14 (39)	0.682
Donor leukocytes, /nl	12.8 (3.5–29.6)	12.6 (2.6–36.0)	0.765	14.3 (6.3–20.0)	12.6 (3.5–29.6)	0.870	12.0 (4.7–28.5)	14.3 (3.7–29.6)	0.110
Donor CrP, mg/l	152 (2–500)	154 (1–1269)	0.910	128 (11–500)	152 (2–368)	0.822	152 (2–500)	160 (6–368)	0.895
Donor BMI, kg/m^2	25 (18–45)	25 (17–49)	0.871	25 (21–31)	25 (18–46)	0.847	25 (18–46)	26 (21–39)	0.154
Donor diuresis last hour, ml	150 (60–500)	150 (35–1000)	0.890	150 (60–500)	150 (60–500)	0.945	135 (60–500)	200 (80–500)	0.064
Donor time on ventilator, days	4 (1–30)	4 (1–30)	0.812	4 (2–14)	5 (1–30)	0.248	4 (1–30)	5 (2–17)	0.187

In a first step, KTRs with BK viremia were treated by reduction of immunosuppression. Calcineurin inhibitor dose was reduced according to trough levels and where necessary MMF/MPA dose was reduced. In a second step, KTRs with BKVN were treated by changing immunosuppression. Tacrolimus was replaced by cyclosporine. MMF/MPA was replaced by azathioprine. Methylprednisolone was maintained unchanged. KTRs who did not respond to the modification of immunosuppression were additionally treated with cidofovir in a first step and IVIG in a second step.

Infection monitoring and prophylaxis

Screening for BKV-load, CMV-load, and EBV-load in serum was performed pretransplantation, monthly until +6 months, then 3 months until +12 months post-transplantation, and yearly thereafter. In addition, screening for BKV-load was performed at any unexplained rise in serum creatinine and in case of acute rejection or antirejection treatment.

All patients with a high-risk CMV constellation (D+R−) received a prophylaxis with valganciclovir for 3 months post-transplantation. Oral prophylaxis for pneumocystis jirovecii pneumonia with trimethoprim/sulphamethoxazole was administered 6 months post-transplantation.

Quantitative PCR for BKV-DNA detection

BKV-load was measured by TaqMan Real Time PCR as described previously [11,12]. Briefly, DNA was isolated from serum using a QIAamp DNA Mini Kit (Qiagen Corp., Hilden, Germany) according to manufacturer's instructions. PCR was based on the TaqMan platform (ABI). PCR amplifications were set up in a reaction volume of 25 u/µl using primer and probe at final concentrations of 900 nM and 5 µM, respectively. Primers and probe were designed to amplify the VP1 region of BKV, respectively. A plasmid standard containing the VP1 coding region of respective virus was used to determine the copy number per ml. Thermal cycling was begun with an initial denaturation step at 95 °C for 10 min that was followed by 40 cycles at 95 °C for 15 s (denaturation) and 60 °C for 1 min (reannealing and extension). The detection level is the lowest viral load measured within the range of linearity, 3000 copies/ml serum.

Statistical methods

Statistical tests were performed using SPSS Version 19 (SPSS, Chicago, IL, USA). For comparisons of study groups, two-sided Mann–Whitney U-test for nonparametric independent samples was used. For comparisons between paired

Table 2. Characteristics of BKV-replication.

	BKV group (n = 103)	BK nephropathy (n = 24)	BK viremia (n = 79)	P value	Early-onset BKV (n = 67)	Late-onset BKV (n = 36)	P value
Transplant age at BK viremia, month	4 (0–73)	4 (1–50)	4 (0–73)	0.684	2 (0–6)	22 (7–73)	–
Duration of BK viremia, month	2 (1–60)	10 (3–60)	1 (1–6)	<0.001	2 (1–60)	1 (1–48)	0.240
1 month, n (%)	50 (49)	–	50 (63)	<0.001	29 (43)	21 (58)	0.155
2–3 months, n (%)	27 (26)	2 (8)	25 (32)	0.032	20 (30)	7 (19)	0.349
4–12 months, n (%)	15 (15)	11 (46)	4 (5)	<0.001	13 (19)	2 (6)	0.079
>12 months, n (%)	11 (11)	11 (46)	–	<0.001	5 (7)	6 (17)	0.186
BKV-load in serum, copies/ml							
BKV-load at diagnosis	1.9×10^4 (3.0×10^3–5.9×10^6)	1.4×10^5 (4.5×10^4–5.9×10^6)	1.1×10^4 (3.0×10^3–2.0×10^5)	<0.001	2.3×10^4 (4.5×10^3–5.9×10^6)	2.2×10^4 (3.0×10^3–7.5×10^5)	0.764
Peak BKV-load	2.9×10^4 (3.0×10^3–1.6×10^7)	6.1×10^5 (7.3×10^4–1.6×10^7)	1.7×10^4 (3.0×10^3–2.0×10^5)	<0.001	2.8×10^4 (4.5×10^3–1.6×10^7)	5.0×10^4 (3.0×10^3–3.5×10^6)	0.178
<10.000 copies/ml, n (%)	39 (38)	–	39 (49)		25 (37)	14 (39)	1
10.000–100.000 copies/ml, n (%)	34 (33)	1 (4)	33 (42)	<0.001	22 (33)	12 (33)	1
>100.000 copies/ml, n (%)	30 (29)	23 (96)	7 (9)	<0.001	20 (30)	10 (28)	1
Recurrence after negativity, n (%)	16 (16)	8 (33)	8 (10)	0.011	9 (13)	7 (19)	0.569
Peak BKV-load after recurrence	8.8×10^3 (4.7×10^3–6.9×10^5)	7.4×10^3 (4.7×10^3–6.8×10^5)	9.4×10^3 (8.1×10^3–1.7×10^4)	–	8.4×10^3 (4.7×10^3–9.4×10^4)	1.7×10^4 (6.1×10^3–6.8×10^5)	–
Time to recurrence after negativity, month	3 (1–18)	3 (2–8)	4 (1–18)	–	3 (1–8)	4 (2–18)	–
Histology							
Presumptive BKVN, n (%)	5 (21)	5 (21)	–	–	3 (4)	2 (6)	–
BKVN only, n (%)	17 (71)	17 (71)	–	–	10 (15)	7 (19)	–
BKVN + acute rejection, n (%)	7 (29)	7 (29)	–	–	6 (9)	1 (3)	–
Pattern A	7 (29)	7 (29)	–	–	5 (7)	2 (6)	–
Pattern B	9 (38)	9 (38)	–	–	7 (10)	2 (6)	–
Pattern C	3 (13)	3 (13)	–	–	1 (1)	2 (6)	–
Concomitant CMV-replication							
CMV-load at diagnosis, copies/ml	2.2×10^3 (1.0×10^3–6.0×10^5)	1.8×10^3 (1.1×10^3–4.4×10^3)	3.2×10^3 (1.0×10^3–6.0×10^5)	–	2.1×10^3 (1.0×10^3–6.0×10^5)	2.7×10^3 (1.1×10^3–4.4×10^3)	–
Peak CMV-load, copies/ml	4.3×10^3 (1.0×10^3–6.0×10^5)	1.8×10^3 (1.1×10^3–4.4×10^3)	1.1×10^4 (1.0×10^3–6.0×10^5)	–	5.4×10^3 (1.0×10^3–6.0×10^5)	2.7×10^3 (1.1×10^3–4.4×10^3)	–
Duration of CMV-replication, month	2 (1–10)	1 (1–2)	3 (1–10)	–	2 (1–10)	1 (1)	–
CMV disease, n (%)	15 (15)	0 (0)	15 (19)	0.019	13 (19)	2 (6)	0.079

Table 2. continued

	BKV group (n = 103)	BK nephropathy (n = 24)	BK viremia (n = 79)	P value	Early-onset BKV (n = 67)	Late-onset BKV (n = 36)	P value
Maintenance immunosuppression							
Tac trough levels at diagnosis, μg/l	7.1 (2.8–18.3)	8.9 (3.8–15.7)	6.9 (2.8–18.3)	0.254	8.6 (2.8–18.3)	5.1 (2.8–12.1)	<0.001
CyA trough levels at diagnosis, μg/l	171 (64–364)	177	164 (64–364)	–	172 (80–364)	84 (64–103)	–
MMF dosing at diagnosis, mg/day	2000 (0–2000)	2000 (1000–2000)	1500 (0–2000)	0.004	2000 (0–2000)	1500 (0–2000)	0.205
Therapeutic intervention, n (%)							
(1) Reduction of CNI	50 (49)	11 (42)	39 (49)	1	40 (60)	10 (28)	0.004
+Reduction of MMF/MP	11 (11)	5 (21)	5 (6)	0.050	7 (10)	4 (11)	1
(2) Switch from Tac to CyA	28 (27)	24 (100)	4 (5)	–	17 (25)	11 (31)	0.645
+switch from MMF/MPA to Aza	24 (23)	20 (83)	4 (5)	–	14 (21)	10 (28)	0.469
+Cidofovir	12 (12)	9 (38)	0	–	8 (12)	4 (11)	1
+IVIG	5 (5)	5 (21)	0	–	3 (4)	2 (6)	1
No intervention, n (%)	36 (35)	0 (0)	36 (46)	<0.001	17 (25)	19 (53)	0.009
Allograft function							
Serum creatinine at diagnosis, mg/dl	1.64 (0.55–4.91)	1.82 (0.79–3.15)	1.61 (0.55–4.91)	0.737	1.65 (0.73–4.91)	1.60 (0.79–4.23)	0.544
Peak serum creatinine, mg/dl	1.96 (0.73–8.02)	2.52 (1.11–8.02)	1.83 (0.73–5.2)	0.004	2.14 (0.73–7.29)	1.84 (0.79–8.02)	0.120
Δ serum creatinine, mg/dl	0.19 (0–5.9)	0.83 (0.05–5.9)	0.11 (0–2.8)	<0.001	0.23 (0–5.8)	0.18 (0–5.9)	0.389
Recovery to baseline creatinine, n (%)	89 (86)	15 (63)	74 (94)	<0.001	62 (93)	27 (75)	0.018
Graft loss due to BKVN, n (%)	3 (3)	3 (13)	0 (0)	–	1 (1)	2 (6)	–

samples, two-sided Wilcoxon signed-rank test for nonparametric dependent samples was used. Outcomes were measured with Kaplan–Meier models and overall strata comparisons measured by log-rank tests. Univariate and multivariate stepwise logistic regression analyses were performed to assess risk for groupings adjusted for age, gender, and maintenance immunosuppression as covariates. Clinical and infectious characteristics were compared across groups using Fisher's exact test or chi-square test for categorical variables, and Student's *t*-test for continuous variables. Box-plots show median, interquartile range, and 95th percentile. Two-sided *P*-values <0.05 were considered statistical significant.

Results

Clinical characteristics and outcomes

Altogether 103 KTRs with BK viremia were analyzed. Median follow-up after transplantation was 79 months (range 5–117 months), during which 14 KTRs died (13.6%) and 12 returned to dialysis (11.6%). Three of 24 KTRs (12.5%) with BKVN showed allograft loss due to BKVN. Compared to the entire study population {52 years [median, range (18–78)], 62% males}, KTRs developing BK viremia {54 years [median, range (18–76)], 69% males} showed no differences for age and gender ($P = 0.693$; $P = 0.235$). Sixty-seven of 103 KTRs (65%) showed early-onset BKV-replication and 36 of 103 KTRs (35%) showed late-onset BKV-replication. While 8.5% of first transplant KTRs showed early-onset BKV-replication and 3.2% of first transplant KTRs showed late-onset BKV-replication, 3.8% of retransplant KTRs showed early-onset and 9.8% of retransplant KTRs showed late-onset BKV-replication ($P = 0.001$).

The control group consisted of 598 KTRs with a median follow-up after transplantation of 55 months (range 12–119). Sixty-eight KTRs died (11.4%) and 42 returned to dialysis (7.0%). Only KTRs who were strictly monitored for BKV were included. Analysis of clinical characteristics of the BK viremia versus control group, BKVN versus BK viremia group, and early-onset versus late-onset BK viremia group is shown in Table 1.

Overall, no differences for patient survival and death-censored allograft survival were observed between KTRs with BKV-replication, KTRs with BK viremia only, KTRs with BKVN, KTRs with early-onset BKV-replication, KTRs with late-onset BKV-replication, and the control group ($P > 0.05$; Fig. 1a and b).

Kidney transplant recipients developing BKV-replication showed significantly worse renal function compared to the control group starting at +72 months post-transplantation (Fig. 2a; $P = 0.015$). KTRs with BKVN showed significantly worse renal function compared to the control group at any time starting at +12 months post-transplantation ($P < 0.05$), and significantly worse renal function compared to KTRs with BK viremia only starting at 60 months ($P < 0.05$). KTRs with BKVN were more likely not to recover to baseline creatinine after BKV-clearance ($P < 0.001$).

Kidney transplant recipients developing late-onset BKV-replication showed significantly worse renal function compared to the control group starting at +60 months post-transplantation (Fig. 2b; $P = 0.015$). KTRs with late-onset BKV-replication showed significantly worse renal function compared to KTRs with early-onset

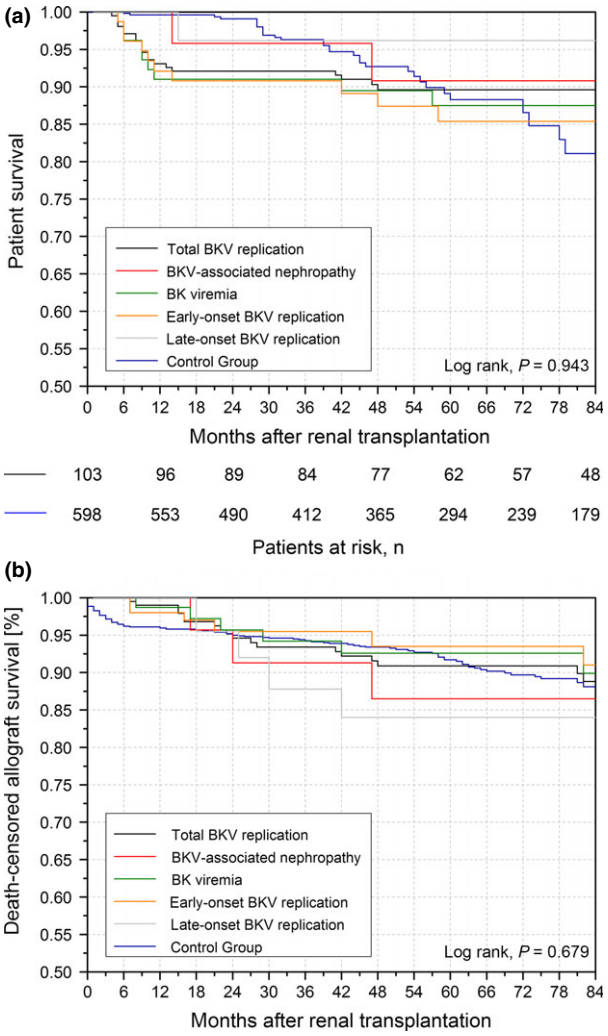

Figure 1 (a) Kaplan–Meier plot of patient survival by BKV-replication after renal transplantation. No differences were observed between KTRs with BKVN, KTRs with BK viremia only, early-onset, late-onset BKV-replication, and the control group (Log rank, $P = 0.943$). (b) Kaplan–Meier plot of death-censored graft survival by BKV-replication after renal transplantation. No differences were observed between KTRs with BKVN, KTRs with BK viremia only, early-onset, late-onset BKV-replication, and the control group (Log rank, $P = 0.979$).

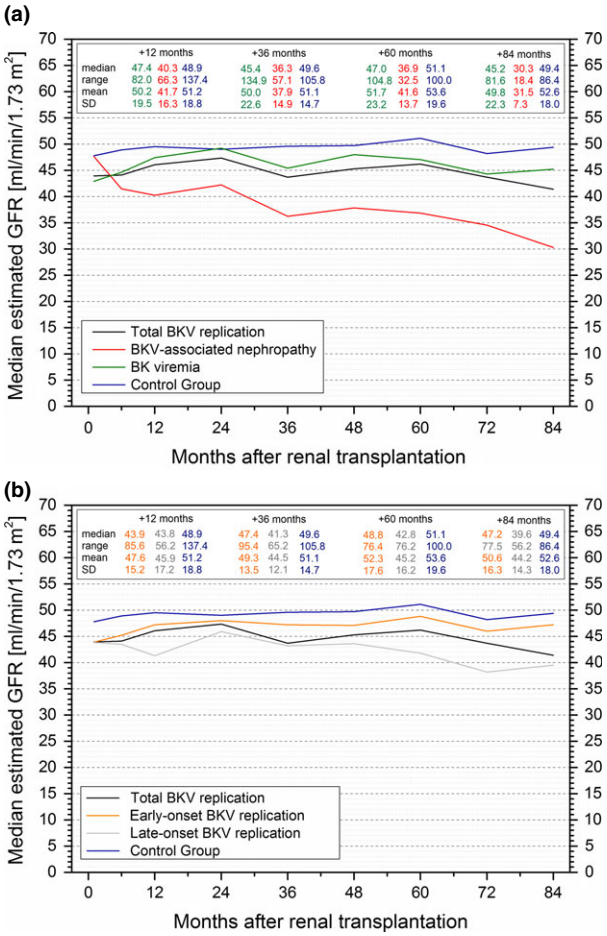

Figure 2 (a) Decreased median eGFR in patients with BKVN ($P < 0.05$). KTRs developing BKV-replication showed significantly worse renal function compared to the control group starting at +72 months post-transplantation ($P = 0.015$). KTRs with BKVN showed significantly worse renal function compared to KTRs with BK viremia only starting at +60 months ($P < 0.05$). (b) Decreased median eGFR in patients with late-onset BKV-replication ($P < 0.05$). KTRs developing late-onset BKV-replication showed significantly worse renal function compared to the control group starting at +48 months post-transplantation ($P = 0.015$). KTRs with late-onset BKV-replication showed significantly worse renal function compared to KTRs with early-onset BKV-replication starting at +12 months ($P < 0.05$).

BKV-replication starting at +12 months post-transplantation ($P < 0.05$). KTRs with late-onset BKV-replication were more likely not to recover to baseline creatinine after BKV-clearance ($P = 0.018$).

Risk factors associated with early-onset BKV-replication versus late-onset BKV-replication and BK viremia versus BKVN

Analysis of clinical and virological characteristics of the BK viremia versus control group, BKVN versus BK viremia group, and early-onset versus late-onset BK viremia group is shown in Tables 1 and 2. Risk factors associated with the development of BKV-replication by multivariate analysis included acute rejection {3.530 [2.278–5.469]; [hazard radio, 95% confidence interval (HR, 95% CI)]; $P < 0.001$}, CMV viremia [2.345 (1.512–3.636); (HR, 95% CI); $P < 0.001$], and lymphocyte-depleting induction [1.940 (1.084–3.472); (HR, 95% CI); $P = 0.026$] after adjusting for age, gender, and maintenance immunosuppression. In this context, the presence of concomitant CMV viremia, concomitant acute cellular rejection, and poor HLA-match distinguished KTRs with early-onset BKV-replication from KTRs with late-onset BKV-replication. KTRs with late-onset BKV-replication were more likely to undergo renal retransplantation and have positive pretransplant panel-reactive antibodies (PRA; $P < 0.05$). This difference was most prominent between KTRs with early-onset BKVN (100% first transplantation) and KTRs with late-onset BKVN (50% retransplantation; $P = 0.007$). No differences in clinical characteristics were observed between KTRs who developed early-onset BK viremia after induction with lymphocyte-depleting agents compared to IL-2R antagonists.

Among KTRs with BK viremia, higher MMF dosing at onset of BKV-replication was identified as an independent risk factor for progression to BKVN ($P = 0.004$).

Virological characteristics in KTRs with early-onset BKV-replication versus late-onset BKV-replication and BK viremia versus BKVN

The highest incidence of BK viremia was observed in the early post-transplant period with 65.0% of cases occurring within the first 6 months (Fig. 3). The median time of diagnosis was 4 months (range 0–73) after renal transplantation. No differences were observed between the onset of BKVN and BK viremia only ($P > 0.05$).

Analysis of virological characteristics of the BKV group, BKVN versus BK viremia group, and early-onset versus late-onset BK viremia group is shown in Table 2.

Discussion

The goal of treatment of active BKV-replication includes elimination of the virus and preserving renal allograft function. Many different therapeutic protocols have been evaluated so far. Among these therapeutic interventions, reduction and modification of immunosuppressive therapy remains the cornerstone in the therapy of BKV-replication despite the risk to induce acute allograft rejection [20–25]. In this context, the evaluation of risk factors in the different settings of BKV-replication is of major importance to optimize BKV-screening and therapeutic interventions.

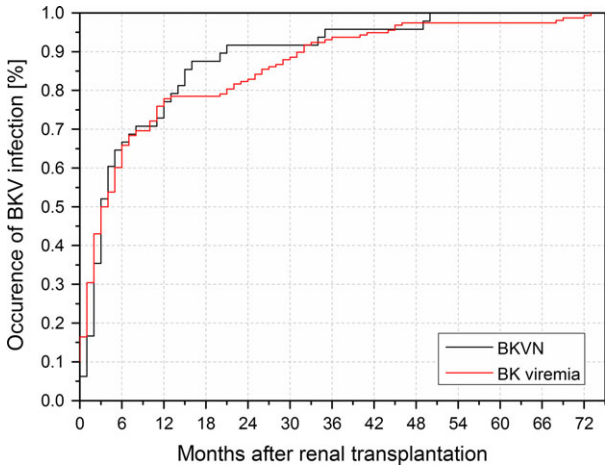

Figure 3 Onset of BK viremia and BKVN after renal transplantation. Sixty-five percent of KTRs showed early-onset BK viremia within the first 6 months post-transplantation.

In this study, we describe the course of 862 single KTRs with regard to BKV-replication to address differences in risk factors, viral kinetics, and outcomes of KTRs with early-onset BKV-replication and late-onset BKV-replication.

Firstly, our results suggest lymphocyte-depleting induction, concomitant CMV-reactivation, and acute cellular rejection as risk factors for early-onset BKV-replication. These observations support previous works, suggesting that the intensity of immunosuppression is the key issue in the pathogenesis of BKV-replication in the early period of high-dose immunosuppression. Here, previous studies suggest an association between lymphocyte-depleting induction and higher incidences of early-onset BKV-replication [26]. However, our data did not show a difference for lymphocyte-depleting induction between KTRs with early-onset and late-onset BKV-replication and therefore question this association. This finding suggests that other factors than lymphocyte-depleting induction may contribute to the increased risk of BK viremia in presensitized KTRs. These factors may include presensitization due to re-transplantation with an increased risk of acute cellular rejection, PRA, and higher CNI trough levels. This may also explain why previous studies, that use lymphocyte-depleting induction in a larger number of KTRs without presensitization compared to our study, show no association of lymphocyte-depleting induction and BK viremia [25].

Our results further indicate that concomitant CMV-reactivation may serve as an important cofactor for BKV-replication. Impaired CMV-specific immunity in a state of overimmunosuppression predisposes patients to early CMV-reactivation which further alters the immunocompromised state. Graft rejection may be accelerated by proinflammatory cytokine release, upregulation of HLA molecules or adhesion during CMV-replication, and therefore necessitate a further increase in immunosuppressive therapy [27,28].

Our data further show that KTRs developing late-onset BKV-replication are more likely to undergo renal retransplantation with the presence of preformed PRA. In addition, a very recent study showed that BKV-replication is associated with allosensitization in terms of any *de novo* donor-specific antibody formation as well as development of *de novo* classes I and II individually [24]. Our own data showed that KTRs developing BKV-replication had significantly higher frequencies of alloreactive T cells in the early post-transplant period. As BKV-replication appears to be a complication affecting almost exclusively recipients of renal transplantation, the intensity of immunosuppression itself cannot be responsible alone [29,30]. Therefore, inflammation within the graft as a result of previous episodes of acute rejections predisposing to allosensitization and a chronic inflammatory state in the presence of donor-specific antibodies can be considered as important risk factors [29,30]. In contrast to some prior studies [5,6], our analysis did not find any association between recipient age and gender. In the context of donor and recipient origin, very recent work provided evidence for BKV donor origin after kidney transplantation using sequencing of the BKV-VP1 typing region in single cases of 20 recipient/donor pairs [31]. Here, it might be of special interest to determine to which extent donor origin contributes to early-onset and late-onset BKV-replication.

Secondly, our data did not identify any clinical risk factors for the progression of BK viremia to BKVN. In this context, our own data showed that KTRs with progression to BKVN were those not able to mount a sufficient BKV-specific T-cell response without reduction of maintenance immunosuppression [32,33]. Moreover, tacrolimus trough levels during the first 12 post-transplant months, measured at the time of BKV detection, were not significantly different between KTRs with BK viremia and KTRs with progression to BKVN. However, those with progression to BKVN showed significantly higher MMF dosing at the time of BKV detection. The tendency for higher tacrolimus trough levels may in addition contribute to higher MMF exposure in these KTRs. Regarding the impact of MMF on BK viremia, previous studies remain controversial [18,24]. Here, the origin of BKV-replication may be of particular importance: KTRs without pre-existing BKV-specific immunity, that develop BK viremia from donor origin, might be particularly impacted by high levels of CNI that impair BKV-specific T-cell activation. In contrast, KTRs with pre-existing BKV-specific immunity, that develop BK viremia from recipient/donor origin due to a loss of protective BKV-specific immunity, might be impacted by high doses of MMF

that impair BKV-specific T-cell expansion/recovery. Our findings of higher MMF dosing in KTRs with progression to BKVN support previous studies suggesting that impaired expansion/recovery of BKV-specific T cells predisposes KTRs to BKVN [32,33].

Thirdly, in line with previous observations [25], our data show no differences in patient survival and allograft survival between KTRs developing BKV-replication, BKVN, and the control group. Interestingly, however, KTRs with BK viremia showed impaired allograft function in long-term follow-up. This finding may be at least in part explained by cases of undiagnosed BKVN in the cohort of KTRs with BK viremia. In addition, this observation may be related to the very recently suggested association of BKV-replication with allosensitization in terms of development of *de novo* donor-specific antibodies [25]. This hypothesis is further strengthened by our observations that KTRs with late-onset BKV-replication show inferior allograft function compared to KTRs with early-onset BKV-replication and are more likely not to return to baseline creatinine after BKV-clearance.

According to our results, future protocols for the therapy of BKV-replication should attempt to evaluate the efficacy of reducing MMF in the early phase of BKV-replication to prevent progression to BKVN. In addition, due to inferior outcomes of renal function in KTRs with late-onset BKV-replication, a more intense screening of BKV-replication needs to be implemented in presensitized KTRs.

Our study has several strengths. We describe the incidence of BK viremia and BKVN in a cohort almost exclusively maintained on tacrolimus, MMF, and methylprednisolone with a standardized immunosuppression reduction approach on BK viremia. Due to our BKV-screening protocol, we are able to show for the first time a clear differentiation between KTRs developing early-onset and late-onset BKV-replication.

Our study also had some limitations. Firstly, although the BK virus screening protocol was instituted prospectively, this study is a single-center retrospective study. Secondly, screening for BK viruria was not performed routinely in our study population, so BK viruria only cannot be addressed among our control group. Thirdly, due to the relatively high detection level for BK viremia of 3000 copies/ml, our study may underestimate the number of KTRs with BK viremia. Fourthly, our study clearly lacks a characterization of the donor as BKV serostatus, BK viruria/viremia, or BKV sequencing in donor/recipient pairs to further address the influence of donor and recipient origin on early-onset BKV reactivation.

In summary, our data compare risk factor profiles of KTRs with early-onset BKV-replication and KTRs with late-onset BKV-replication. While more intensified immunosuppression is associated with early-onset BKV-replication, a chronic inflammatory state in presensitized KTRs undergoing retransplantation may contribute to late-onset BKV-replication. Due to inferior allograft outcomes in KTRs with late-onset BKV-replication, a more intensified BKV-screening needs to be implemented in presensitized KTRs.

Authorship

TS: participated in data collection, writing of the study, the performance of the research and data analysis. NB: participated in data collection and research design. PR: participated in research design, writing of the study, the performance of the research and in data analysis.

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Supporting Information

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

Table S1 Patient characteristics: early-onset versus late-onset BK viremia and BK nephropathy.

Table S2 Patient characteristics: early-onset versus late-onset BK viremia and BK nephropathy.

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