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Prospective Monitoring of Polyomavirus BK Replication and Impact of Pre-Emptive Intervention in Pediatric Kidney Recipients

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Polyoma BK virus (BKV)-associated nephropathy (PVAN) is a relevant cause of poor renal allograft survival. In a prospective analysis, we monitored BKV DNA in blood and urine samples from 62 consecutive pediatric kidney recipients. In patients with BKV replication, we analyzed the impact of reduction of maintenance immunosuppression on viral load kinetics and PVAN in patients with BKV replication. BKV-specific immunity was concomitantly evaluated on blood samples of viremic patients, by measuring the frequency of BKV-specific interferon-γ-producing and cytotoxic T cells, and BKV IgG antibody levels. At a median follow-up of 24 months, BK viruria was observed in 39 of 62 patients, while BK viremia developed in 13 patients (21%). In all viremic patients, immunosuppression reduction resulted in the clearance of viremia, and prevented development of PVAN, without increasing the rate of acute rejection or causing graft dysfunction. As a consequence of immunosuppression adjustment, an expansion of BKV-specific cellular immunity was observed that coincided with viral clearance. We conclude that treating pediatric kidney transplant patients pre-emptively with immunosuppression reduction guided by BKV DNA in blood is safe and effective to prevent onset of PVAN. BKV-specific cellular immunity may be useful to guide this intervention.

Key words: Cellular immunity, humoral immunity, pediatric kidney transplantation, polyomavirus BK, preemptive therapy

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Introduction

Polyomavirus BK (BKV) replication has been identified as an important cause of interstitial nephritis and graft loss in recipients of a renal allograft (1–4). Current therapeutic options for BKV-associated nephropathy (PVAN) are limited. No available anti-viral drug has proven successful in controlled trials, and control of viral infection is tentatively obtained by means of reduction of immunosuppression (5–9). However, therapeutic intervention instituted at a late stage of allograft involvement has shown only a marginal beneficial effect (8–9). More recently, increased awareness and improved diagnostic techniques have led to treatment at earlier stages, before significant renal allograft dysfunction, reducing the incidence of graft loss (7,10).

Pre-emptive reduction of immunosuppression in patients at high risk of developing PVAN could represent an optimal strategy to avoid onset of established nephritis. It has been demonstrated that positive PCR testing for BKV DNA in plasma is a sensitive and specific method for early identification of viral nephropathy, and the appearance of viremia has been described to precede the onset of PVAN (2,7). Thus, positivity for BKV viremia could identify a subset of kidney recipients with pre-clinical disease that would benefit from a pre-emptive intervention. A recent study has shown that pre-emptive reduction of immunosuppression in adult renal transplant recipients with positive plasma BKV DNA was associated with resolution of viremia and absence of PVAN development without an increased risk for acute rejection or graft loss (11). However, current standard of care is treatment of established PVAN (4), and further clinical evidence is needed for pre-emptive intervention to be considered as the strategy of choice in the management of kidney recipients with BKV replication.

Caution against adopting a pre-emptive strategy to avoid progression from BKV replication to overt PVAN comes from the notion that reestablishment of a functional cellular immunity to BKV by reduction of maintenance immunosuppression may facilitate the onset of acute rejection, or cause direct damage to the affected kidney. It has

lately been hypothesized that development of BKV-capsid protein (VP1)-specific CD8 T-cell response in allograft recipients with BKV plasma replication may be associated with negative PVAN outcome (12). However, prospective data on BKV-specific immunity are lacking in patients with positive BKV viremia or PVAN, and, therefore, the role of BKV-specific T cells on the course of BKV infection and pathogenesis of PVAN remains to be clarified.

We performed a prospective analysis to assess the incidence and kinetics of BKV replication in pediatric kidney transplant recipients, and evaluated the impact of protocol or pre-emptive reduction of immunosuppression on viral load kinetics, BKV-specific immunity and the course of PVAN.

Patients and Methods

Study population

Between January 2002 and November 2005, 68 patients referred to the Pediatric Nephrology Unit, G. Gaslini Institute-Genova received kidney transplantation and posttransplantation care, and were enrolled in this prospective study. Five patients were excluded for poor protocol compliance, and 1 for development of posttransplant lymphoproliferative disease, leaving 62 patients available for analysis.

This study was conducted according to Institutional guidelines, and patients or legal representatives signed an informed consent before entering the study.

Transplantation management

Baseline immunosuppression protocols included induction with the anti-CD25 monoclonal antibody basiliximab, and double or triple therapy with either cyclosporine-A (CyA) or tacrolimus associated to prednisone alone or prednisone and mycophenolate mofetil (MMF). Three highly sensitized patients received induction with anti-tymocyte globulin (ATG). CyA was started at 10 mg/kg/day in two divided doses, targeting whole blood 2-h post-dose levels (C2) of 1400–1800 ng/mL. Progressive reduction of CyA was applied from month +2, to reach C2 target levels of 1200–1600 ng/mL during the second posttransplant month, 1000–1400 ng/mL through months +3 to +6, and 800–1200 ng/mL thereafter. Tacrolimus was started at 0.3 mg/kg/day in two divided doses, targeting whole blood through levels of 12–16 ng/mL. Progressive reduction of tacrolimus was started from month +2, to reach through target levels of 10–14 ng/mL and 8–12 ng/mL during the second and third posttransplant month, 6–10 ng/mL through months +4–+6, and 5–8 ng/mL thereafter. MMF was administered at 800 mg/m^2/day in two divided doses for the first posttransplant month, and reduced to 600 mg/m^2/day from month +2.

Standard of care was to perform a renal biopsy for graft dysfunction defined as an elevation of creatinine to >20% of baseline. Treatment of acute rejection consisted of pulse steroids and, in case of steroid resistance (n = 1), photopheresis and switch to tacrolimus were employed as rescue therapy.

BKV monitoring

Blood and urine samples were collected at +1, +3, +6, +9, +12, +18, +24, +36 and +48 months after transplantation, during routine laboratory tests. A total of 458 samples were collected and stored from 62 patients, with a sampling range of 5–13, according to the length of follow-up. BK viruria was defined as presence of BKV DNA in urine, while BK viremia was defined by the detection of viral DNA in serum. Patients found positive for BK viremia underwent a renal biopsy to evaluate the presence of PVAN. Baseline BK serology at transplantation was evaluated for all patients.

Virologic methods

Polymerase chain reaction methods: Qualitative PCR for detection of BKV DNA was used for patient management. DNA was extracted from serum, urine and biopsy samples by RNA EXTRA kit (GeneDia, Napoli, Italy). BKV DNA detection was performed by a previously described nested, qualitative, PCR assay (13). In the first amplification reaction, a sequence of BKV DNA, 748 bp long, within the noncoding transcription control region, was amplified by the outer primers BKTT1 and BKTT2 (AAGGTCCATGAGCTCCATGGATTCTTCC and CTAGGTCCCCCAAAAGTGCTAGAGCAGC, respectively). The second amplification reaction amplified a sequence 386 bp long, within the first, by the primers BK1: GGCCTCAGAAAAAGCCTCCACACCCTTACTACTTGA and BK2: CTTGTCGTGACAGCTGGCGCAGAAC. The specificity of the second PCR products was then determined by restriction analysis with the enzymes Bsu36I and SacI. Samples found positive for BKV DNA with the nested PCR, were quantitated by a BKV-specific real-time PCR, according to a previously reported method (7). Briefly, BKV DNA was isolated with QIAamp DNA Mini Kit (Qiagen, Milan, Italy) and quantified by real-time PCR (TaqMan/7700, Stratagene Mx4000 or BioRad iCycler [BioRad, Milan, Italy]). The following primers were used for BKV-LT amplification: BKV forward; AGCAGGCAAGGGTTCTATTACTAAAT (26-mer), BKV reverse; GAAGCAACAGCAGATTCTCAACA (23-mer), Fam-tamra-labelled BKV probe; AAGACCCTA AAGACTTTCCCTCTGATCTACACCAGTTT (38-mer). Primers and probes were obtained from Eurogentec (Geneva, Switzerland). The linear range of the real-time PCR is 10e2–10e8 cp/mL and the limit of detection was 300 copies (cp)/mL.

Serology methods: Patients' serostatus was determined testing 1:400 diluted plasma in an ELISA format for IgG with 50 ng of BK virus-like particles as antigens coated to solid phase purified from VP1 expressing baculovirus-infected SF9 cells after lysis and gradient centrifugation. A cut-off of OD492 nm >0.110 after subtraction of non-VLP expressing lysates was considered positive (S Bodaghi and HH Hirsch, unpublished results).

BKV pre-emptive therapy

In patients with stable renal function, defined as <15% increase from baseline serum creatinine concentration, maintenance immunosuppression was reduced according to the standard protocol detailed in *Transplantation Management*, as a first step. In case of stable renal function and increasing viral load over the next 4 weeks, calcineurin inhibitor was reduced of 15–20%, according to plasma levels, as a second step. If BK viremia persisted, MMF was halved as a third step or discontinued as a fourth step.

Patients with presumptive PVAN (4) and worsening renal function, in addition to protocol reduction of maintenance immunosuppression, were treated with pre-emptive reduction of calcineurin inhibitor (15–20%, according to plasma levels), as a first step. In case of increasing viral load over the next 4 weeks, reduction of calcineurin inhibitors was followed by halving of MMF dosage as a second step, and MMF discontinuation as a third step in case of further increase in viral load.

One patient with presumptive PVAN and concomitant acute rejection was first treated with pulse steroids for rejection, and subsequently received pre-emptive intervention, as described above.

BKV-specific immune monitoring

All samples were processed within 24 h from blood draw, and cryopreserved. Immunological monitoring of a single patient was performed by thawing and plating all samples on the same day in a single assay.

BKV-specific T-cell immunity was evaluated by measuring the frequency of virus-specific IFNγ-secreting cells in a ELISPOT assay, and cytotoxicity of BKV-specific cell cultures, using a 51-chromium release assay. Peripheral blood mononuclear cells (PBMC) were collected at different time points and cultured for 8–10 days in the presence of 15-mer peptide pools spanning the entire BKV-VP1 and large T (LT) proteins (0.5 μg/mL concentration of each single peptide, JPT Peptide Technologies, Berlin, Germany).

For ELISPOT assays, 96 well multiscreen filter plates (MAIPS 4510, Millipore, Bedford, MA) were coated with 100 μL of primary antibody (IFN-γ, Mabtech, Nacka, Sweden) at 2.5 μg/mL, and incubated overnight at 4°C. Cultured T cells were seeded in the absence or presence of VP1 and LT peptide mixes, 0.5 μg/mL). After incubation for 24 h at 37°C, 100 μL of biotinylated secondary antibody (Mabtech, 0.5 μg/mL) was added, and plates were then processed according to standard procedure. IFN-γ-producing spots were counted using an Elispot reader (Bioline, Torino, Italy) (14). The number of spots per well was calculated after subtracting assay background, quantitated as an average of 24 wells containing only complete medium, and specific background, quantitated as the sum of cytokine spots associated with responders alone.

Specific cytotoxic activity was assessed by standard ^{51}Cr-release assay, against a panel of targets including autologous phytohemagglutinin (PHA) blasts pulsed for 2 h with 2 μg/mL of VP-1 and LT peptide mix or with 2 μg/mL of control peptide (EBV-LMP2 peptide mix, JPT), and incubated overnight with ^{51}Cr (100 μCi). In brief, PBMC cultured for 8–10 days with VP-1 and LT peptide pools were incubated with 1000 target cells at E:T ratios of 20:1, 10:1, 5:1, 2.5:1. To obtain PHA blasts, PBMC were cultured in the presence of PHA (4 μg/mL) for 3–6 days.

Statistical analysis

Data were expressed as mean ± SD or as median and range, as appropriate.

The correlation of clinical parameters with BKV viruria and viremia was evaluated by Student's *t*-test. The contribution of various risk factors to BKV replication was evaluated by chi-square test. The p-values <0.05 were considered statistically significant; p-values from 0.05 to 0.1 were not considered significant, but reported in detail, while p-values >0.1 were reported as NS. Statistical analysis was performed using the NCSS System (NCSS, Cary, NC).

Results

Incidence and timing of BKV

Sixty-two renal transplant recipients were prospectively followed for a median of 24 months (range, 12–60). The clinical characteristics of the patients are summarized in Table 1.

Table 1: Characteristics of the patients enrolled into the study

Age (years), median (range)	15 (2–25)
Sex, M/F	37/25
No. of transplant	
1st transplant	47
retransplant	15
Donor type, deceased /living	59/3
Donor age (years), median (range)	13 (1–57)
Baseline immunosuppression	
Any regimen + anti-CD25 mAb	59
Any regimen + ATG	3
CyA–based regimen	49
FK–based regimen	13
Any regimen + MMF	53
BKV serology, neg/pos	10/52
DGF	7
Acute rejection	9
CMV replication	27
EBV DNA positivity	31

CyA = cyclosporine-A; FK = tacrolimus; MMF = mycophenolate mofetil; DGF = delayed graft function.

BKV urinary shedding was observed in 39 of the 62 patients, at a median onset of 3 months (range, 1–24). BK viremia was detected in 13 of the 62 kidney recipients, at 1–18 months (median, 3 months): in 4 patients, viruria preceded viremia by 3.5 months (range,1–17 months) while 9/13 patients showed concomitant detection of viruria and viremia. No PVAN was observed at 1 year, and none had been detected at the last date of observation, with a median observation period of 24 months. Kaplan–Meier analysis showed an estimated incidence of BK viruria at 2 years of 64% (95% confidence interval, 53–78%) and a probability of BK viremia at 2 years of 22% (95% confidence interval, 13–35%) (Figure 1).

BKV urine levels varied over a wide range, the median peak level observed being 2.8×10^7 (range, 1×10^4–3.2×10^{10}). Compared to kidney recipients who did not develop viremia, patients with plasma BKV replication had higher peak urine levels (2.6×10^6 vs. 2.1×10^9), and a longer duration of viruria (Table 2). BKV load in plasma reached a median peak level of 2.2×10^4 (range, 3.1×10^3–2.9×10^5). A longer duration of viremia was observed in patients with higher peak plasma loads (Table 2).

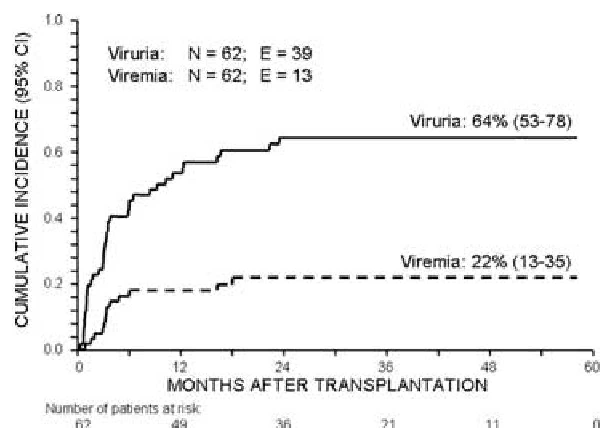

Figure 1: Cumulative risk of developing polyomavirus BK viruria and viremia in pediatric recipients of kidney transplantation. N = total number of patients; E = events (number of patients developing viruria or viremia).

Table 2: BK virus parameters of the patients enrolled into the study

	BKV replication				p-Value		
	Viruria alone		Viremia		Viruria alone vs. viremia		
Parameters (median values)	Single positive sample[1] (n = 13)	Sustained positivity[2] (n = 13)	Single positive sample[3] (n = 6)	Sustained positivity[4] (n = 7)	Total (n = 13)	Single positive sample (n = 6)	Sustained positivity (n = 7)
Time KTx to viruria (months)	9 m	3 m	2 m	3 m	p = 0.08	p = NS	p = NS
Time KTx to viremia (months)	–	–	4 m	3.5 m			
Duration of viruria	3 m	6 m	5.5 m	11 m	$p < 0.05$	p = NS	$p < 0.05$
Duration of viremia	–	–	1 m	4 m			
Viruria relapses	3	2	1	2			
Peak viruria (genomes/mL)	2.36×10^5	3.8×10^7	4.96×10^9	2.07×10^9	$p < 0.005$	$p < 0.05$	$p < 0.001$
Peak viremia (genomes/mL)	–	–	6.04×10^3	1.70×10^5			

[1]Single positive sample = single positive sample on the 3-monthly screening.
[2]Sustained positivity = two or more consecutive positive samples on the 3-monthly screening.
[3]Single positive sample = single positive sample on the monthly screening.
[4]Sustained positivity = two or more consecutive positive samples on the monthly screening.

BKV replication and correlation with clinical parameters

Relation of BKV urinary shedding and BKV replication to patients' transplant data was evaluated in a univariate analysis. No differences in age at transplant, sex, living donor graft use, previous graft number, number and class of matched donor-recipient HLA alleles, and development of cytomegalovirus or Epstein–Barr virus replication, existed between patients with or without evidence of BK viruria and viremia. There was no significant association between any specific immunosuppressive agent, recipient BKV serological status, delayed graft function (DGF) and presence of BK viruria or viremia. A trend toward an increased risk of developing BK viremia was observed in patients experiencing an acute rejection episode (p = 0.06).

There was no difference in the serum creatinine levels at 1, 3, 6, 12, 24 and 36 months between the groups of patients who developed BK viremia and/or viruria and those who remained negative.

BKV replication and effect of reduction of immunosuppression

Reduction of immunosuppression was associated with clearance of viremia in all patients (Table 3). The median time to viremia clearance was 2 months (range 1–8 months). Twelve of 13 viremic patients cleared viruria within the observation period of the study, with a median time to clearance of 8 months (range 2–30 months). One of the latter patients experienced a relapse of viremia 11 months after plasma BK clearance, while two patients showed viruria relapse 3 months after urine BK clearance.

Among the allograft recipients with a single plasma positivity (n = 6), five patients cleared BK viremia following protocol reduction of immunosuppression, while one patient, who became positive at +18 months, required a 15% decrease of calcineurin inhibitor dosage (Table 3). Among the 7 kidney recipients with BK replication of longer duration, clearance of BK viremia through protocol reduction of immunosuppression was only reached in two patients, while in the other five a pre-emptive reduction of immunosuppression was applied. Four of the five latter patients, who reactivated within the sixth posttransplant month, showed a slight deterioration of renal function, which prompted pre-emptive reduction of immunosuppression. Calcineurin inhibitor was reduced in three of four patients, followed by MMF reduction in two patients (Table 3). The presence of leukopenia at the time of BK reactivation prompted reduction/discontinuation of MMF, rather than calcineurin inhibitor, in the fourth patient. The fifth patient belonging to this group, who did not show deterioration of renal function, was treated with pre-emptive reduction of immunosuppression due to late viremia appearance (+18 months).

Two patients showed viremia and concomitant acute cellular rejection (Banff type: IA), and were treated accordingly. No patient experienced acute rejection as a consequence of reduction of immunosuppression prompted by BK viremia positivity.

Immunological evaluation of patients with BK viremia

BKV-specific immunity was evaluated on samples obtained from the patients at different time points after transplantation, and results were then analyzed in relation to BKV status of the patient at the time of evaluation. BKV-specific immune response of healthy age-matched BKV-seropositive donors (n = 12) was also evaluated.

When analyzed at the nearest time before reactivation, the patients who developed BK viremia had a low frequency of BKV-IFNγ-producing cells (BKV-VP1 response, median and range: 25, 0–129 spots/10^5 PBMC; BKV-LT response, median and range: 39, 0–131 spots/10^5 PBMC), compared to the healthy seropositive control group (BKV-VP1 response, median and range: 101, 28–242 spots/10^5

Table 3: Pre-emptive treatment of BK viremia

UPN	Age at KTx (years), Sex	BKV serology	Viremia onset (day)	Urine BKV-load at onset (cp/mL)	Plasma BKV-load at onset (cp/mL)	S-crea at onset (μmol/L)*	IS reduction	Plasma BKV-load at reduction (cp/mL)	BK viremia duration (months)	Urine BKV-load at f-up (cp/mL)	S-crea f-up (μmol/L))
Single positivity**											
03008047	8, M	neg	88	3.47E + 10^6	2.91E + 10^4	99	protocol reduction***	<1000	1	<2500	99
03019128	7, M	pos	60	3.22E + 10^{10}	3.13E + 10^3	82	protocol reduction	<1000	1	1.50E + 10^4	80
03022906	17, M	neg	221	1.42E + 10^7	3.89E + 10^3	109	protocol reduction	<1000	1	<2500	103
04022542	6, M	neg	27	2.12E + 10^{10}	7.77E + 10^3	76	protocol reduction	<1000	1	<2500	71
04034726	24, F	pos	548	9.85E + 10^9	2.18E + 10^4	74	↓ CI (−15%)	<1000	1	6.10E + 10^5	80
05002193	13, M	pos	164	7.26E + 10^7	4.31E + 10^3	144	protocol reduction	<1000	1	<2500	139
Sustained positivity											
03017126	18, M	pos	523	3.30E + 10^8	2.11E + 10^5	133	↓ CI (−15%)	<1000	2	<2500	130
03019514	23, M	pos	59	7.95E + 10^8	2.46E + 10^3	**141*******	protocol reduction and ↓ CI (−20%) + ↓ MMF (−50%) switch CyA-sirolimus*****	2.31E + 10^5 <1000	4	<2500	113
03024966	9, M	pos	96	8.10E + 10^8	5.36E + 10^3	62	protocol reduction	<1000	4	<2500	70
03036365	13, M	pos	97	1.42E + 10^{10}	6.84E + 10^4	**98**	protocol reduction and ↓ MMF (−50%)****** ↓ MMF (−100%)	6.8E + 10^4 <1000	4	<2500	75
04000102	10, F	pos	108	2.07E + 10^9	6.66E + 10^3	73	protocol reduction	<1000	5	<2500	62
04009398	25, F	pos	142	1.00E + 10^8	6.45E + 10^4	**106**	protocol reduction and ↓ CI (−20%) ↓ MMF (−50%) ↓ MMF (−50%)	1.5E + 10^5 1.9E + 10^5 <1000	8	<2500	80
05007184	24, M	pos	110	1.00E + 10^9	1.70E + 10^5	**174**	protocol reduction and ↓ CI (−15%)	<1000	4	2.34E + 10^4	165

* Values in bold font represent patients with increased serum creatinine at the time of viremia.

** Single positivity = single positive sample on the monthly screening; sustained positivity = two or more consecutive positive samples on the monthly screening.

*** Reduction of maintenance immunosuppressive drugs according to the institutional protocol for transplant management, as described in **Patients & Methods**, section *Transplantation Management*.

**** The patient presented with BK viremia and concomitant acute rejection. He was first treated with pulse steroids, and then proceeded to reduction of maintenance IS.

***** Due to concomitant treatment of acute rejection, the patient halved MMF in addition to CI reduction. Moreover, as a second step, switch from calcineurin inhibitor to rapamycin was applied.

****** The patient presented with leukopenia. Thus, reduction/discontinuation of MMF, rather than calcineurin inhibitor, was applied.

S-crea = serum creatinina; IS = immunosuppression; CI = calcineurin inhibitors; MMF = mycophenolate mofetil; CyA = cyclosporine-A.

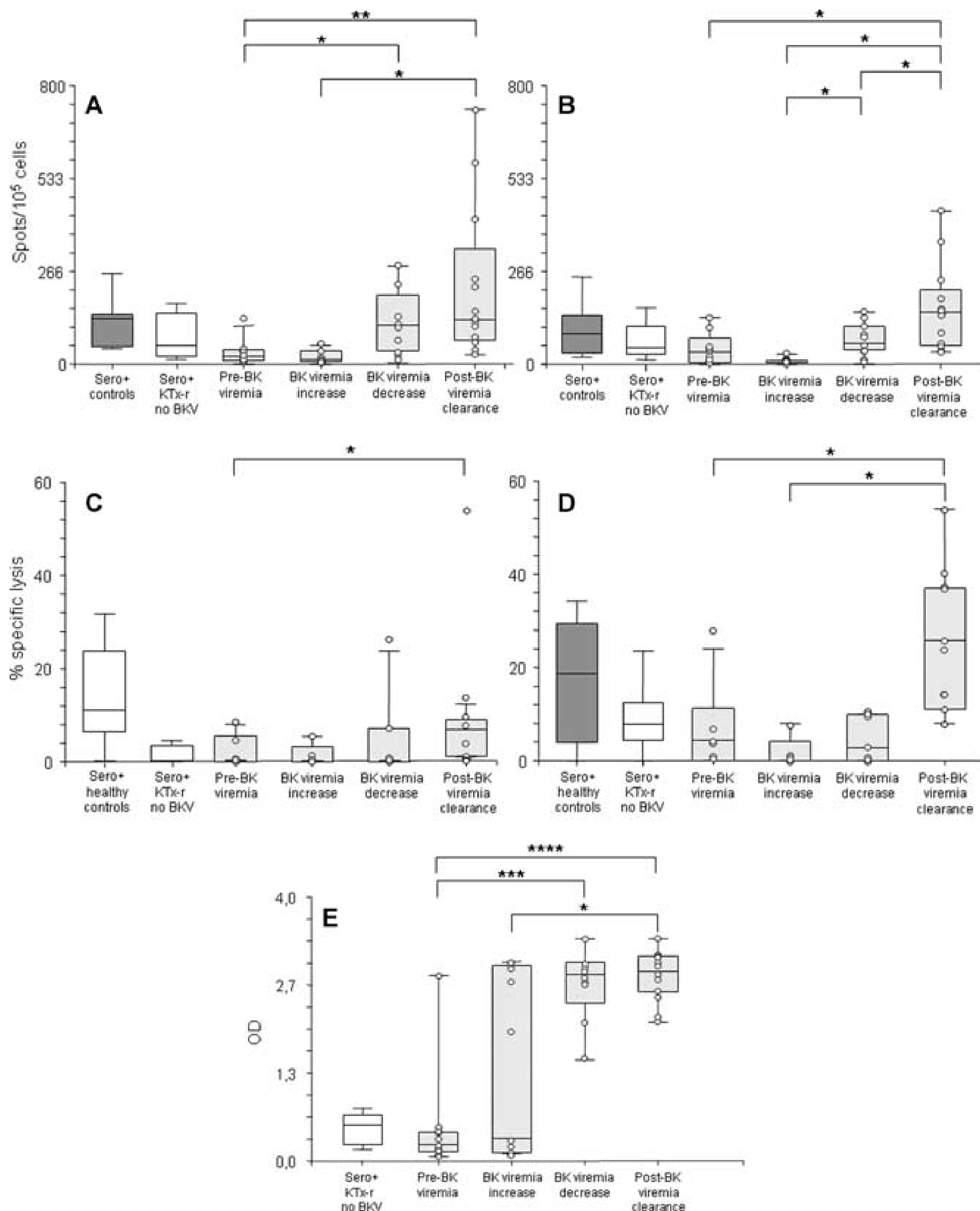

Figure 2: Prospective evaluation of BKV-specific cellular and humoral immune response in the viremic patients treated with reduction of immunosuppression. Data on the frequency of IFNγ-secreting lymphocytes (panels A and B) and virus-specific cytotoxicity (panels C and D), measured in patients' cultured PBMC, obtained at the earliest time point before development of viremia, at viremia increase, at decrease and after viremia clearance, after 10-day stimulation with VP1 (IFNγ-secreting lymphocytes = panel A; cytotoxicity = panel C) and LT (IFNγ-secreting lymphocytes = panel B; cytotoxicity = panel D) peptides, are reported. As control, responses of healthy BKV-positive individuals (dark-grey bars) and of BKV-seropositive kidney transplant recipients who did not develop BK viremia and/or viruria (evaluation at 1–2 months, white bars) are reported. IFNγ-secreting cells are represented as number of spots/10^5 cells (mean spots of triplicate experiments). Cytotoxicity is represented as % specific lysis at an effector to target ratio of 10:1 (mean of triplicate experiments). In panel E, data on patients' anti-BKV IgG at the different time points before, during and after BK viremia are reported. As control, responses of BKV-seropositive kidney transplant recipients who did not develop BK viremia and/or viruria (evaluation at 1–2 months, white bars) are reported. All results are reported as median and quartiles. Differences among results obtained at different time points were analyzed by Wilcoxon test (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.005$).

PBMC; BKV-LT response, median and range: 81, 10–243 spots/10^5 PBMC). These values were found to be further decreased at the time of viral reactivation (median response, BKV-VP1: 16 spots/10^5 PBMC, range: 0–54; BKV-LT: 7 spots/10^5 PBMC, range: 0–29) (Figures 2A and B). After reducing immunosuppression, viremic kidney recipients started to mount BKV-specific immune responses, that reached levels comparable or higher than those displayed by healthy controls upon attainment of viral clearance (BKV-VP1 response, median and range: 128, 27–730 spots/10^5 PBMC; BKV-LT response, median and range: 150, 36–434 spots/10^5 PBMC). Longitudinal analysis of exemplificative patients is shown in Figure 3. Since CD8+ T cells and cytotoxic function are thought to be crucial for clearance of virus-infected cells, we then proceeded to evaluate the cytotoxic capacity of BKV-specific T cells present in the patients' lines. PBMC collected after viral clearance and stimulated for 8–10 days with BKV antigens displayed measurable lytic activity against autologous PHA blasts pulsed with BKV-VP1 and LT, compared with cells recovered before viral reactivation (BKV-VP1 response, mean cytotoxicity at a 5:1 effector to target ratio: 8%, range 1–14 vs. 0%, range 0–9; BKV-LT response, mean cytotoxicity at a 5:1 effector to target ratio: 26%, range 8–54 vs. 5%, range 0–24) (Figures 2C and D).

Evaluation of humoral immunity to BKV showed that the median IgG EIA levels before viral reactivation, or in the early phase of viremia, were significantly lower than those observed at the time of BKV clearance (median EIA units, before BK viremia: 0.26, range 0.11–0.43, after BK viremia clearance: 2.84, range 2.05–3.05) (Figure 2E).

Discussion

Despite the multitude of studies performed in adult kidney recipients, there is limited information regarding BKV infection in pediatric kidney recipients. The studies reported to date are retrospective, or evaluate limited prospective sampling (13,15–17). In our prospective study, the prevalence of BK viruria and viremia are 63% and 21%, respectively. The prevalence observed is almost twice that of retrospective data in pediatric patients (13,16–17) and prospective results in adult kidney recipients (7,11). In the case of other pediatric studies, the augmented incidence is likely ascribable to the increased probability of detecting BKV replication by prospective sampling. Regarding the prospective studies in adults, the higher prevalence cannot be solely explained by the longer duration of follow-up (12 months vs. a median of 24 months), but may reflect the increased viral reactivation risk experienced by pediatric recipients, due to the use of more intense immunosuppression. Finally, the increased prevalence might also be determined by the fact that all patients in our cohort had ureteral stents (11).

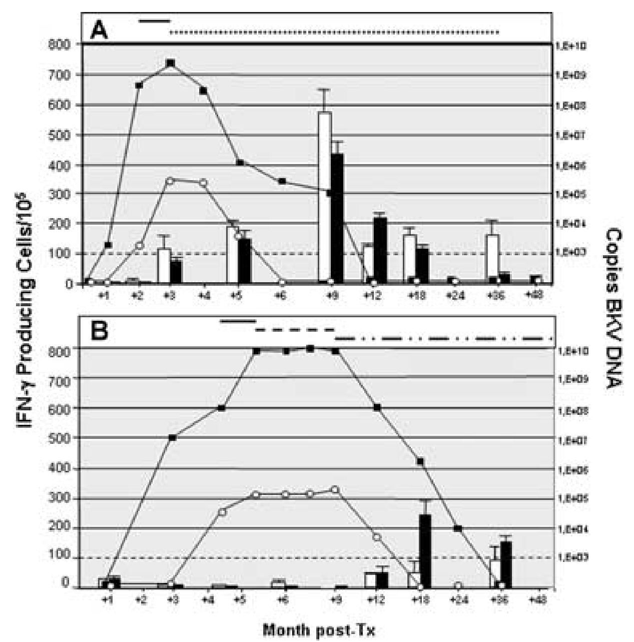

Figure 3: Kinetics of viral load and BKV-specific cellular immune response in two patients with sustained viremia treated with immunosuppression reduction. Horizontal axis in each frame = time course in months after renal transplantation. Upper frame in each panel = modification of maintenance immunosuppression (continuous line = protocol reduction + ↓ 20% calcineurin inhibitor in panel B and protocol reduction + ↓ 20% calcineurin inhibitor + ↓ 50% MMF in panel A; dotted line = switch calcineurin inhibitor-sirolimus; broken line = ↓ 50% MMF; broken + dotted line = ↓ 50% MMF). Lower frame in each panel: BKV-specific IFN-γ secreting cells (spot forming units/10^5 cells) (vertical axes on the left side; bars) and viral load (vertical axes on the right side; copies/μL) (empty circles = viremia; filled squares = viruria). The frequency of IFNγ-secreting lymphocytes, measured in the cultured PBMC of patients #03019514 (Panel A) and #04009398 (Panel B), after 8–10 day stimulation with VP1 (white bars) and LT (black bars) peptide pools, are reported (mean spots of triplicate experiments ± SD).

Regarding the analysis of risk factors, we could only observe a trend toward an augmented risk of BKV reactivation in patients who had experienced an episode of acute rejection. Differently from our retrospective study (13), we could not confirm the role of recipient pretransplant BKV serology on the risk of BK viruria posttransplant. This observation may be due to both the lower number of evaluated patients (100 vs. 62), and different methodology employed to evaluate serology. In particular, the EIA method used in this study has a higher sensitivity. Moreover, the quality of antibody response measured by hemoagglutination inhibition assay and EIA assay is different.

Notwithstanding early diagnosis of patients at risk for PVAN, and timely treatment, which have improved short-term outcome (7,10,18), PVAN may continue to pose a threat to long-term renal allograft survival (9,19). It was

recently suggested that treatment of patients with BKV viremia, a pre-requisite for progression to PVAN (7), could abrogate the development of PVAN. In a prospective BKV surveillance/intervention study, enrolling 200 adult kidney recipients, Brennan et al. found that pre-emptive withdrawal of immunosuppression in patients with BK reactivation led to resolution of viremia, without progression to PVAN and increase in the rate of acute graft rejection (11). Importantly, at 12-months follow-up, renal function in the treated patients was comparable to that observed in patients who never became viremic (11). The results of our study confirm that, also in the pediatric setting, a strategy of monitoring for BKV replication and applying pre-emptive reduction of immunosuppression in viremic patients is associated with plasma BKV clearance, without significant risk of precipitating acute rejection or allograft dysfunction. The absence of PVAN cases in this cohort followed prospectively compares favorably with a 5% rate of PVAN, and one graft loss, observed in our historical cohort (13). The outcome of patients enrolled in our prospective study suggests that a surveillance program on a 3-monthly basis in the first year posttransplant is sufficient to identify patients at risk and prevent PVAN development. Extending the surveillance beyond the first posttransplant year may be useful to identify allograft recipients with late onset BK viremia. These patients could be at a particular risk of progression to PVAN, since in routine clinical practice they are not likely to undergo unprompted further modification of immunosuppressive drug levels. Regarding the choice of a strategy for maintenance immunosuppression modification, we elected to start by cautious adjustments in the levels of calcineurin inhibitors, rather than reducing or discontinuing MMF as a first step. This decision was prompted by the notion that pediatric recipients have a higher risk of acute rejection compared to their adult counterpart (20), and that calcineurin inhibitors may play a role as cofactors in the establishment of graft damage that predisposes to PVAN (21). Moreover, a recent report has underscored the impact of calcineurin inhibitor dose reduction in stabilization of renal function in PVAN patients (9).

Prospective evaluation of BKV-specific immune response was prompted by the still open question of whether an increase of specific immunity in viremic patients could be beneficial or, rather, could worsen the patients' outcome by inducing kidney damage. It showed that levels of virus-specific T cells and IgG antibodies were low or absent before viral replication, and significantly increased with declining BK viremia subsequent to reduced immunosuppression. The peak of both cellular and humoral BKV-directed immune responses coincided with BK viremia resolution, and was followed by long-term good allograft function. These data are in line with previous observations from our and other groups (14,22–25) suggesting that the expansion of specific immunity to BKV has a protective, rather than a pathogenetic role, in the development of PVAN. It is noteworthy that we also detected BKV VP1- and LT-specific cytotoxic responses in our patients after viral clearance. Since their presence was associated with maintenance of good graft function, these cytotoxic T cells did not seem to constitute a threat to allograft survival. The apparent contrast of our data to a previous observation (12), reporting the onset of PVAN and graft loss in the only 2 patients, of 15 analyzed, with detectable BKV-specific CD8+ T-cell responses in the peripheral blood, may be explained by sampling times. Indeed, in a patient who lost the graft to PVAN in our historical cohort (13), emergence of BKV-directed cellular immunity was observed, coincident with a therapeutic reduction in maintenance immunosuppression, that could not influence graft outcome in an advanced stage of kidney damage (26). Our study suggests that emergence of BKV-specific immune response in viremic patients without kidney involvement leads to viral clearance and optimal transplant outcome. It cannot be ruled out that a late intervention may contribute to graft dysfunction, by promoting cytotoxic-inflammatory damage at the infection site. Likewise, allospecific T-cell responses, driven by BKV replication, may occasionally emerge in the affected allograft, and create a condition not dissimilar to a rejection episode (12,27).

The assessment of BKV-specific immunity may be a useful tool, in conjunction with the use of blood BKV DNA measurement, to guide therapeutic intervention in kidney recipients with BK viremia or PVAN. The best immunological parameter for this purpose has not yet been defined. In our viremic patients, IgG antibodies directed to BKV rose in the early stages of viremia, when immunosuppression had not yet been adjusted, while BKV-specific T cells expanded at later stages, upon modification of maintenance immunosuppression. Thus, evaluation of cellular immunity likely represents the most valuable strategy in this setting. In this view, an exhaustive analysis of the complete cohort could allow to estimate the clinical utility of immunological monitoring for BKV, and, together with viral load analysis, contribute to a better definition of risk for the patients.

Our study indicates that the use of a pre-emptive strategy to avoid progression from BKV replication to overt PVAN is a feasible and safe option for pediatric recipients of renal transplantation.

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