Transplant International

ORIGINAL ARTICLE

No clinical benefit of rapid versus gradual tapering of immunosuppression to treat sustained BK virus viremia after kidney transplantation: a single-center experience

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SUMMARY

Immunosuppressive drug tapering is currently the recommended treatment of BK virus (BKV) viremia after kidney transplantation; however, its exact modalities remain unclear. We retrospectively compared two consecutive strategies in 111 patients with sustained viremia: a gradual monitoring/tapering group (GT, $n = 57$) before 2012 and a rapid monitoring/tapering group (RT, $n = 54$) after 2012. At viremia diagnosis, the dose of mycophenolic acid (MPA) and tacrolimus levels (T_0) were similar among patient groups. However, following onset, the dose of MPA at 1 month ($P = 0.002$) and 3 months ($P = 0.005$) and Tac T_0 at 1 month ($P = 0.030$) and 3 months ($P = 0.006$) were lower in the RT group. This rapid minimization shortened BKV viremia ($P < 0.001$) and resulted in a better protection of graft function in patients with confirmed BKV-associated nephropathy ($P = 0.033$) without impacting 5-year graft survival. Survival without rejection was similar ($P = 0.571$), but the RT group had increased the development of *de novo* donor-specific antibodies (dnDSAs; $P < 0.001$). Multivariate Cox analysis identified basiliximab versus Thymoglobulin® induction [hazard ratio (HR), 3.090; $P = 0.001$] and the RT strategy (HR, 6.021; $P = 0.002$) as independently associated with dnDSAs. Compared to a gradual tapering, rapid immunosuppression tapering to treat sustained BKV viremia does not improve medium-term clinical outcome but increases the risk of developing dnDSAs.

Transplant International 2019; 32: 481–492

Key words
BK virus, immunosuppression, kidney transplantation

Received: 14 September 2018; Revision requested: 22 October 2018; Accepted: 13 December 2018; Published online: 11 January 2019

doi:10.1111/tri.13392

Introduction

In the absence of effective antiviral drugs, BK virus (BKV) reactivation is a major challenge following kidney transplantation (KT). BKV reactivation most commonly occurs during the first year after transplantation. BKV viruria occurs in 30–50% of kidney transplant recipients (KTRs) with potential progression to BKV viremia in 10–30% of recipients [1–5] and biopsy-proven BKV-associated nephropathy (BKVAN) in 1–10% of patients [6,7]. BKVAN carries serious clinical consequences with an increased risk of graft dysfunction and graft loss [8–10]. Based on OPTN data, graft loss because of BKV or preceded by treatment for BKV ranges from 7% to 8% [11].

The growing clinical challenge of BKV replication after KT led to several guidelines to guide BKV monitoring and management. BKV surveillance after KT is now universally recommended [12–14] to facilitate early diagnosis, develop early therapeutic interventions, and, hopefully, reduce the consequences of BKV reactivation on graft dysfunction and/or graft loss [15–18]. However, the optimal frequency and approach to surveillance for BKV reactivation remain unclear and differ between guidelines [12–14].

Immunosuppressive drug tapering is currently the recommended treatment of BKV reactivation [12]; however, its exact modalities also remain unclear. Moreover, immunosuppression reduction carries risks, including *de novo* donor-specific antibodies (dnDSAs) and graft rejection [19–23].

In 2012, we implemented a profound modification of our strategy for monitoring and treating BKV reactivation, such that kidney transplant recipients (KTRs) underwent a more rapid protocol of immunosuppression tapering than in the past to comply with new recommendations [12–14]. The aim of this single-center, retrospective study is to compare these two different strategies after BKV viremia in a well-phenotyped population of KTRs and to assess the impact of those strategies on virological, immunological, and allograft outcomes.

Materials and methods

Study population

This study includes all kidney transplant recipients treated with an immunosuppressive regimen consisting of tacrolimus (Tac), mycophenolic acid (MPA), and steroids who experienced a sustained BKV viremia (defined as two consecutive positive tests for BKV viremia) between April 2007 and January 2016.

All data regarding donor and recipient were extracted from the DIVAT clinical prospective cohort (Official website: www.divat.fr). Each patient from the present study has given written informed consent to be included in the DIVAT database. IRB approval was not required for this retrospective analysis of standard clinical practice.

Immunosuppression

Our standard immunosuppressive regimen consisted of induction therapy with either rabbit antithymocyte globulin [rATG, Thymoglobulin®, Sanofi, France, (n = 71)] for patients with preformed DSAs and those who underwent retransplantation or basiliximab [Simulect®, Novartis Pharma AG, Basel, Switzerland, (n = 37)] for the others.

Additionally, KTRs considered at increased immunological risk also received four courses of intravenous immunoglobulin (IVIg) post-transplant with or without plasma exchanges and rituximab (Mabthera®; Roche Pharmaceuticals, Basel, Switzerland), as previously reported [24]. Maintenance immunosuppression consisted of a triple-drug regimen (Tac, MPA and steroids) at the time of BKV viremia.

Screening and management of BKV reactivation

All patients received the same clinical follow-up (one visit per week for the first 3 months, followed by one visit every 2 weeks for 3 months, and finally, one visit per month up to 2 years post-transplantation). All had blood sampled during each visit and all biological samples were processed in the same analytical laboratory. In addition, all patients underwent a protocol allograft biopsy at 3 months and 12 months post-transplantation or in the case of graft dysfunction.

Blood BKV viral load was monitored using BKV quantitative real-time PCR (BK Virus R-gene, BioMérieux®, Marcy l'Etoile, France), with a positive threshold value of 2.7 $\log_{10}$ copies/ml in plasma samples.

We modified our local protocol of blood BKV viral load monitoring and BKV viremia management in January 2012. During both periods, sustained viremia, with or without BKVAN, triggered the reduction in immunosuppression. Before 2012, BKV viremia was monitored at 3 and 12 months post-transplantation and in cases of allograft dysfunction. While viremic, KTRs had a more gradual reduction in their immunosuppression (referred to as the gradual tapering group [GT group], consisting of a stepwise reduction every month until BKV viral load decreased in two consecutive steps of 50% MPA

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dose reduction before complete withdrawal and then Tac reduction to reach trough levels (T_0) between 3 and 5 ng/ml). After 2012, BKV viremia was monitored every month during the first year, once at 2 years, and in cases of allograft dysfunction. While viremic, KTRs had a more rapid reduction in their immunosuppression (same stepwise reduction but made every 2 weeks) until BKV viral load decreased [referred to as the rapid tapering group (RT group)]. No patient of this study received a specific antiviral treatment (e.g., cidofovir).

As per our protocol, screening biopsies were systematically performed at 3 and 12 months after transplantation, and indication allograft biopsies were performed in case of allograft dysfunction. All biopsies performed in patients with concomitant BKV viremia were reviewed by two investigators (MR, AV) with systematic SV40 immunohistochemical staining performed (anti-SV40 T Antigen Mouse mAb (PAb416), Calbiochem®, United States). Renal allograft biopsies were classified using the Banff 2007 update of the Banff 1997 classification [25].

Donor-specific antibody

Donor-specific antibody screening, performed using the single antigen flow bead assay (One Lambda, Canoga Park, CA) on the Luminex platform, was systematically performed at 3 and 12 months after transplantation and then annually and in case of indication biopsy.

All circulating antihuman leukocyte antigen (HLA) antibody assessments were performed in one laboratory (Saint-Louis Hospital, Paris). Anti-HLA antibody screenings performed immediately after the administration of IvIg were excluded to prevent false-positive results. Beads showing a normalized mean fluorescence intensity (MFI) greater than 500 were considered positive in our laboratory. For dnDSAs with an MFI between 500 and 1000, we concluded positivity only if the same DSA was still present in a second sample performed at least 2 months after the first positive serum.

Statistical methods

The results are presented as the median and interquartile range (25th–75th percentile) for continuous variables. Frequencies of categorical variables are presented as numbers and percentages. For statistical comparison of the two groups, we used the Mann–Whitney test or Fisher's exact test, when appropriate.

The Kaplan–Meier method was used to estimate the cumulative incidence of events. Survival time started on the date of the first BKV viremia. Survival differences were calculated using the log-rank test. Survival was censored at 5 years after the first positive BKV viremia, at recipient death, or at the last visit before September 2017.

Cox proportional hazards models were fit to quantify the hazard ratios and 95% confidence intervals for the factors associated with post-BKV viremia DSA development. The associations of donor, recipient, transplant parameters, and immunological factors with the event of interest were first assessed in univariate regression analyses. All variables with a P value ≤ 0.20 were then included in one multivariate Cox model.

Analyses were performed with R software (version 3.1.3.) and GraphPad Prism (version 5.00; GraphPad Software, San Diego, CA).

Results

Patients

A total of 111 patients, transplanted between April 2007 and August 2015, were included. Their baseline characteristics are summarized in Table 1. Twenty-four percent of the included patients underwent retransplantation, and 57 (52%) patients had preformed DSAs. All but three patients had received induction therapy with either Thymoglobulin® ($n = 71$) or basiliximab ($n = 37$). In addition, 74 (67%) KTRs considered at increased immunological risk received four courses of intravenous immunoglobulin. DSA-positive patients with MFI >1000 at day 0 ($n = 35$) also received prophylactic rituximab therapy ($n = 23$) together with plasma exchanges or only plasma exchanges ($n = 9$).

Depending on the strategy of BKV viremia monitoring/management, the initial cohort was divided into a GT group ($n = 57$) and an RT group ($n = 54$). Both groups were similar for most baseline characteristics (Table 1). However, recipient and donor ages were older in the RT group ($P = 0.004$ and $P = 0.003$, respectively).

Importantly, the immunological risk profile at transplantation (i.e., including incidence of retransplantation and of patients transplanted with preformed DSAs) and the overall level of immunosuppression received before the onset of BKV viremia were similar.

Monitoring of BKV viremia after kidney transplantation

Blood BKV viral load monitoring was less intensive in the GT group compared to the RT group; the first BKV viremia screening was performed later in the GT group

Table 1. Characteristics of the cohort.

Parameters	Total population (n = 111)	Gradual tapering group (n = 57)	Rapid tapering group (n = 54)	P value
Recipient characteristics				
Age, median (IQR), year	49 (35–60)	43 (35–53)	58 (36–65)	0.004
Gender male, n (%)	68 (61%)	33 (58%)	35 (65%)	0.559
First transplantation, n (%)	84 (76%)	39 (68%)	45 (83%)	0.180
Causes of end-stage renal disease				
Primary glomerulonephritis, n (%)	18 (16%)	11 (19%)	7 (13%)	0.444
Secondary glomerulonephritis, n (%)	8 (7%)	5 (9%)	3 (6%)	0.717
Interstitial nephritis, n (%)	10 (9%)	7 (12%)	3 (6%)	0.323
Diabetes, n (%)	11 (10%)	3 (5%)	8 (15%)	0.118
Hypertension, n (%)	10 (9%)	4 (7%)	6 (11%)	0.520
Cyst/hereditary/congenital, n (%)	32 (29%)	14 (25%)	18 (33%)	0.402
Uncertain, n (%)	22 (20%)	13 (23%)	9 (17%)	0.480
Preformed DSA with MFI >1000, n (%)	35 (32%)	14 (25%)	21 (40%)	0.104
Transplant and donor variables				
Age, median (IQR), year	53 (41–66)	51 (39–56)	58 (46–73)	0.003
Deceased donor, n (%)	91 (83%)	48 (84%)	43 (81%)	0.802
ECD, n (%)	45 (51%)	18 (39%)	27 (63%)	0.034
Cold ischemia time*, median (IQR), h	18 (14.26)	18 (14–24)	18 (14–26)	0.958
Delayed graft function, n (%)	32 (29%)	18 (32%)	14 (26%)	0.675
Immunosuppression				
Induction				
Thymoglobulin, n (%)	71 (64%)	37 (65%)	34 (63%)	0.846
Basiliximab, n (%)	37 (33%)	19 (33%)	18 (33%)	1
None, n (%)	3 (3%)	1 (2%)	2 (4%)	0.612
Tac/MPA/steroids, n (%)	111 (100%)	587 (100%)	54 (100%)	1
Rituximab, n (%)	23 (21%)	12 (21%)	11 (20%)	1
Intravenous immunoglobulin, n (%)	74 (67%)	35 (61%)	39 (72%)	0.314
Plasma exchange, n (%)	31 (28%)	16 (28%)	15 (28%)	1
Follow-up time, median (IQR), days	1858 (1409–2590)	2552 (2192–2942)	1533 (1175–1791)	<0.001

DSA, donor-specific antibody; ECD, expanded criteria donor; IQR, interquartile range; MFI, mean fluorescence intensity; MPA, mycophenolic acid; n, number; Tac, tacrolimus.

*In deceased donor grafts only.

[87 (72–93) vs. 39 days (30–49), $P < 0.001$], and the number of blood BKV viral load assessments during the first post-transplant year was also lower [3 (2–10) vs. 15 (12–20), $P < 0.001$].

Characteristics of BKV reactivation

Compared to the RT group, the GT group had more days to the first diagnosis of BKV viremia after transplantation [133 (86–370) vs. 99 days (74–181), $P = 0.042$] and a lower peak BKV viral load [4.6 (4–5.6) vs. 5.4 $\log_{10}$ copies/ml (4.2–6.5), $P = 0.022$]. Strikingly, the duration of detectable BKV viremia was longer in the GT group [384 (197–521) vs. 105 days (71–240), $P < 0.001$]. Importantly, the number of blood BKV viral load screenings in viremic patients after the diagnosis of BKV reactivation was similar in both groups [10 (5–13) in the GT group vs. 7 (4–14) in the RT group, $P = 0.460$].

The incidence of biopsy-proven BKVAN was identical in the two groups (33%, $P = 1$). Of the 37 biopsies that had led to the diagnosis of BKVAN, six were protocol biopsies (3 in the GT group vs. 3 in the RT group, $P = 1$) and 31 were indication biopsies (16 in the GT group vs. 15 in the RT group, $P = 1$). The causes of indication biopsies were the detection of BKV viremia associated with allograft dysfunction in 15 cases (6 in the GT group vs. 9 in the RT group, $P = 0.289$), the detection of BKV viremia without allograft dysfunction in seven cases (1 in the GT group vs. 6 in the RT group, $P = 0.037$), and allograft dysfunction without the knowledge of BKV viremia in nine cases (9 in the GT group vs. 0 in the RT group, $P < 0.001$).

Immunosuppressive drug management after the diagnosis of BKV viremia

While the daily dose of MPA was similar at the diagnosis of BKV viremia in both groups ($P = 0.103$), the MPA daily doses 1 and 3 months after the first BKV viremia were lower in the RT compared to the GT group ($P = 0.002$ and $P = 0.005$, respectively; Fig. 1a). In addition, the time to first MPA dose reduction after the first BKV viremia was delayed in the GT group compared to the RT group [26 (3–70) vs. 14 days (5–31), $P = 0.063$].

Values for Tac T_0 were similar at the onset of BKV viremia in both groups ($P = 0.917$). However, Tac T_0 values 1 and 3 months after the first BKV viremia were lower in the RT compared to the GT group ($P = 0.030$ and $P = 0.006$, respectively; Fig. 1b).

Graft outcome

Table 2 summarizes the progression of renal allograft function after BKV viremia diagnosis in the entire cohort of viremic patients ($n = 111$), in the restricted group of patients without biopsy-proven BKVAN ($n = 74$), and in the restricted group of patients with biopsy-proven BKVAN ($n = 37$).

Considering the whole cohort of viremic kidney recipients ($n = 111$), baseline eGFR, eGFRs at viremia, 1 month, 3 months, 6 months, and 12 months post-onset of BKV viremia were similar in the GT and the RT groups. A minimal difference was observed for the evolution of eGFR 12 months after viremia diagnosis (ΔeGFR), with a slightly more pronounced decrease in graft function in the GT group compared to the RT group, without

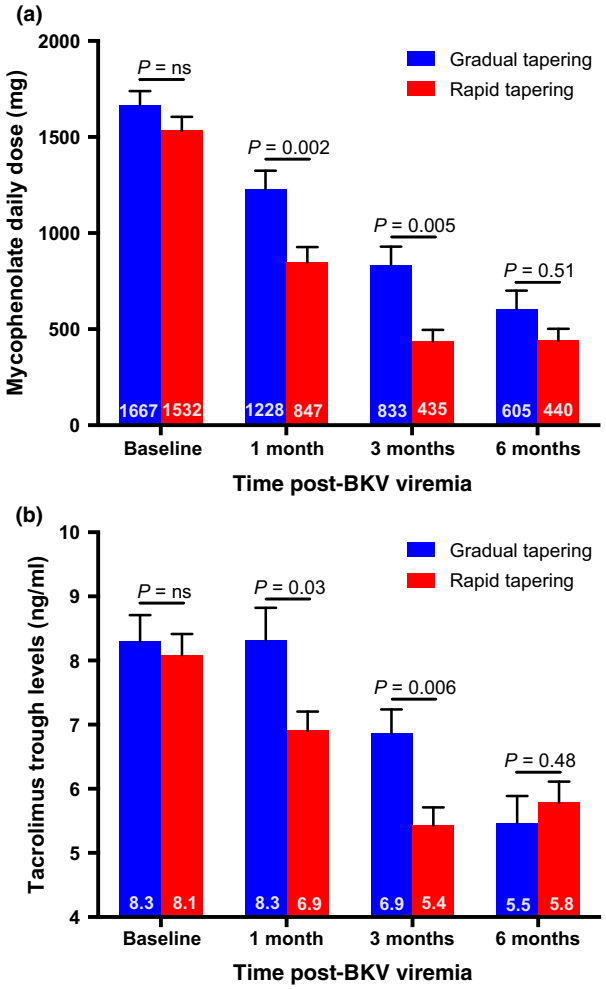

Figure 1 Evolution of the maintenance immunosuppressive regimen after the first detectable BKV viremia. Evolution of the mycophenolic acid daily dose (a) and of the tacrolimus trough levels (b) at different time points following the first detectable BKV viremia. Data are presented as the mean±SEM values. P-values by Mann–Whitney test.

Table 2. Outcomes after sustained BKV viremia.

Parameters	Total population	Gradual tapering	Rapid tapering	*P* Value
Whole cohort of viremic patients	*N* = 111	*N* = 57	*N* = 54	
eGFR at baseline*, median (IQR), ml/min/1.73 m²	72 (56–100)	76 (59–97)	72 (56–103)	0.682
eGFR at viremia, median (IQR), ml/min/1.73 m²	52 (38–69)	49 (37–72)	54 (41–68)	0.656
eGFR at 1 month, median (IQR), ml/min/1.73 m²	53 (39–70)	51 (38–72)	53 (41–67)	0.820
eGFR at 3 months, median (IQR), ml/min/1.73 m²	49 (37–67)	46 (35–67)	50 (38–67)	0.610
eGFR at 6 months, median (IQR), ml/min/1.73 m²	48 (37–67)	46 (36–69)	49 (38–65)	0.767
eGFR at 12 months, median (IQR), ml/min/1.73 m²	54 (34–69)	54 (35–69)	53 (34–68)	0.996
ΔeGFR at 6 months†, median (IQR)	−0.30 (−0.42 to 0.14)	−0.33 (−0.52 to 0.14)	−0.30 (−0.37 to 0.16)	0.321
ΔeGFR at 12 months‡, median (IQR)	−0.26 (−0.47 to 0.11)	−0.37 (−0.49 to 0.17)	−0.21 (−0.43 to 0.05)	0.060
Viremic patients with confirmed BKVAN	*N* = 37	*N* = 19	*N* = 18	
eGFR at baseline*, median (IQR), ml/min/1.73 m²	71 (58–84)	67 (59–81)	72 (58–103)	0.391
eGFR at viremia, median (IQR), ml/min/1.73 m²	48 (38–61)	39 (36–48)	55 (46–66)	0.008
eGFR at 1 month, median (IQR), ml/min/1.73 m²	48 (36–63)	38 (31–55)	53 (48–67)	0.014
eGFR at 3 months, median (IQR), ml/min/1.73 m²	41 (34–59)	39 (28–44)	48 (37–62)	0.085
eGFR at 6 months, median (IQR), ml/min/1.73 m²	41 (27–49)	37 (20–42)	49 (36–58)	0.025
eGFR at 12 months, median (IQR), ml/min/1.73 m²	40 (28–60)	31 (20–44)	50 (33–65)	0.037
ΔeGFR at 6 months†, median (IQR)	−0.38 (−0.57 to 0.3)	−0.56 (−0.68 to 0.33)	−0.32 (−0.42 to 0.3)	0.025
ΔeGFR at 12 months‡, median (IQR)	−0.43 (−0.55 to 0.26)	−0.52 (−0.66 to 0.35)	−0.4 (−0.47 to 0.11)	0.033
Viremic patients without BKVAN	*N* = 74	*N* = 38	*N* = 36	
eGFR at baseline*, median (IQR), ml/min/1.73 m²	76 (56–106)	81 (59–110)	68 (56–101)	0.314
eGFR at viremia, median (IQR), ml/min/1.73 m²	56 (39–73)	61 (40–79)	52 (38–68)	0.352
eGFR at 1 month, median (IQR), ml/min/1.73 m²	56 (42–73)	61 (44–77)	53 (39–66)	0.234
eGFR at 3 months, median (IQR), ml/min/1.73 m²	56 (38–72)	59 (38–73)	50 (39–70)	0.485
eGFR at 6 months, median (IQR), ml/min/1.73 m²	56 (41–69)	64 (43–73)	50 (41–65)	0.109
eGFR at 12 months, median (IQR), ml/min/1.73 m²	61 (43–71)	61 (50–77)	54 (41–68)	0.169
ΔeGFR at 6 months†, median (IQR)	−0.24 (−0.37 to 0.13)	−0.23 (−0.40 to 0.06)	−0.25 (−0.35 to 0.14)	0.701
ΔeGFR at 12 months‡, median (IQR)	−0.22 (−0.42 to 0.08)	−0.29 (−0.44 to 0.08)	−0.19 (−0.26 to 0.06)	0.334

BKVAN, BK virus-associated nephropathy; eGFR: estimated glomerular filtration rate using the Modification of Diet in Renal Disease formula; IQR, interquartile range.

*Baseline eGFR is defined as the highest eGFR from transplantation to the onset of BKV viremia.

†(eGFR at 6 months – eGFR at baseline)/eGFR at baseline.

‡(eGFR at 12 months – eGFR at baseline)/eGFR at baseline.

reaching statistical significance ($P = 0.060$). No difference with regard to graft function evolution was observed in the restricted group without biopsy-proven BKVAN. However, in the patients with biopsy-proven BKVAN, if baseline eGFR was similar in both groups, eGFRs at the onset of BKV viremia and 1 month, 6 months, and 12 months post-viremia were worse in the GT group compared to the RT group ($P = 0.008$, $P = 0.014$, $P = 0.025$, and $P = 0.037$, respectively). The relative decrease in eGFR at 6 and 12 months post-BKV viremia was also more pronounced in the GT group compared to the RT group ($P = 0.025$ and $P = 0.033$, respectively).

Kaplan–Meier analyses of post-BKV viremia acute rejection-free survival (Fig. 2a), death-censored graft survival (Fig. 2b), and patient survival (Fig. 2c) demonstrated no association with the minimization strategy ($P = 0.571$, $P = 0.951$, and $P = 0.760$, respectively, log-rank test). Importantly, even in the patients with biopsy-proven BKVAN, Kaplan–Meier analysis of post-BKV viremia death-censored graft survival also demonstrated no association with the minimization strategy ($P = 0.613$, log-rank test).

Emergence of *de novo* DSAs after BKV viremia

The number of Luminex® tests that were performed during the 1500 days following the first BKV viremia diagnosis was similar in both groups [4 (2–5) in the GT group vs. 4 (3–5) in the RT group, $P = 0.799$]. The time from BKV viremia diagnosis to the emergence of dnDSAs was shorter in the RT group than in the GT group [640 (205–887) vs. 1326 days (845–2007), $P = 0.006$], as also illustrated by our Kaplan–Meier analysis showing an accelerated development of dnDSAs in the RT group compared to the GT group ($P < 0.001$, log-rank test; Fig. 2d).

The median MFI of the dnDSAs was similar in both groups [2350 (1371–15042) vs. 1300 (804–1710) in the GT and the RT groups, respectively, $P = 0.130$], most of them being class II dnDSAs (67% vs. 59% in the GT and the RT groups, respectively, $P = 0.652$).

Univariate Cox analysis showed that recipient age at transplantation, the type of induction treatment, a treated acute rejection before the first detectable BKV viremia, and the immunosuppression minimization strategy after BKV viremia were associated ($P < 0.2$) with dnDSAs development (Table 3). The multivariate Cox analysis identified basiliximab induction compared to Thymoglobulin® [hazard ratio (HR), 3.090; 95% confidence interval (CI), 1.285–7.430; $P = 0.001$] and the RT strategy compared to the GT strategy (HR, 6.021; 95% CI, 1.902–19.067; $P = 0.002$) as independently associated with dnDSAs development (Table 3).

Discussion

We demonstrate that an early and intensive monitoring of BKV viremia associated with a rapid tapering of immunosuppression to treat sustained BKV viremia leads to an increased incidence of dnDSAs but does not seem to be associated with a difference in medium-term clinical outcome compared to a strategy of less intensive monitoring and gradual tapering of immunosuppression.

Based on new guidelines [12], we profoundly modified our clinical management of BKV reactivation in 2012. As a consequence, our retrospective analysis of two periods, before and after these profound modifications, provides fresh insights into the optimal frequency of blood BKV viral load monitoring and the clinical management of BKV viremia by immunosuppression minimization. These insights are important because existing guidelines do not provide specific guidance as to the exact frequency of BKV screening and the pace of immunosuppression tapering.

Early and frequent screening for BKV reactivation assumes that an early diagnosis leading to a rapid intervention would improve graft outcomes [15–18]. The expectation that early treatment of BKV reactivation will limit the consequences of BKVAN on graft histology and function is logical, but in the absence of specific antiviral agents that have proven efficacious in the clinical setting, the only recommended treatment is to reduce immunosuppression, a strategy that may be even more risky when it occurs early after transplantation. By increasing the frequency of blood BK viral load screening, we diagnosed BKV reactivation 1 month earlier than we had in the past—at a median time of 99 days post-transplantation. In this regard, a first screening at 3 months, as suggested by the AST guidelines, is in line with the findings of our study [12]. However, if a more intensive screening led to more rapid detection of BKV viremia, it did not impact the incidence of BKVAN and the medium-term allograft outcome. Nevertheless, one of the key findings of our study is that, compared to a less intensive monitoring and a gradual tapering of immunosuppression, an intensive monitoring of BKV viremia led to the diagnosis of BKVAN before parenchymal damage and severe allograft dysfunction. Furthermore, diagnosis when BKV-induced graft injury remains minimal is associated with a better preservation of graft function after BKVAN diagnosis, with no impact, however, on medium-term allograft survival.

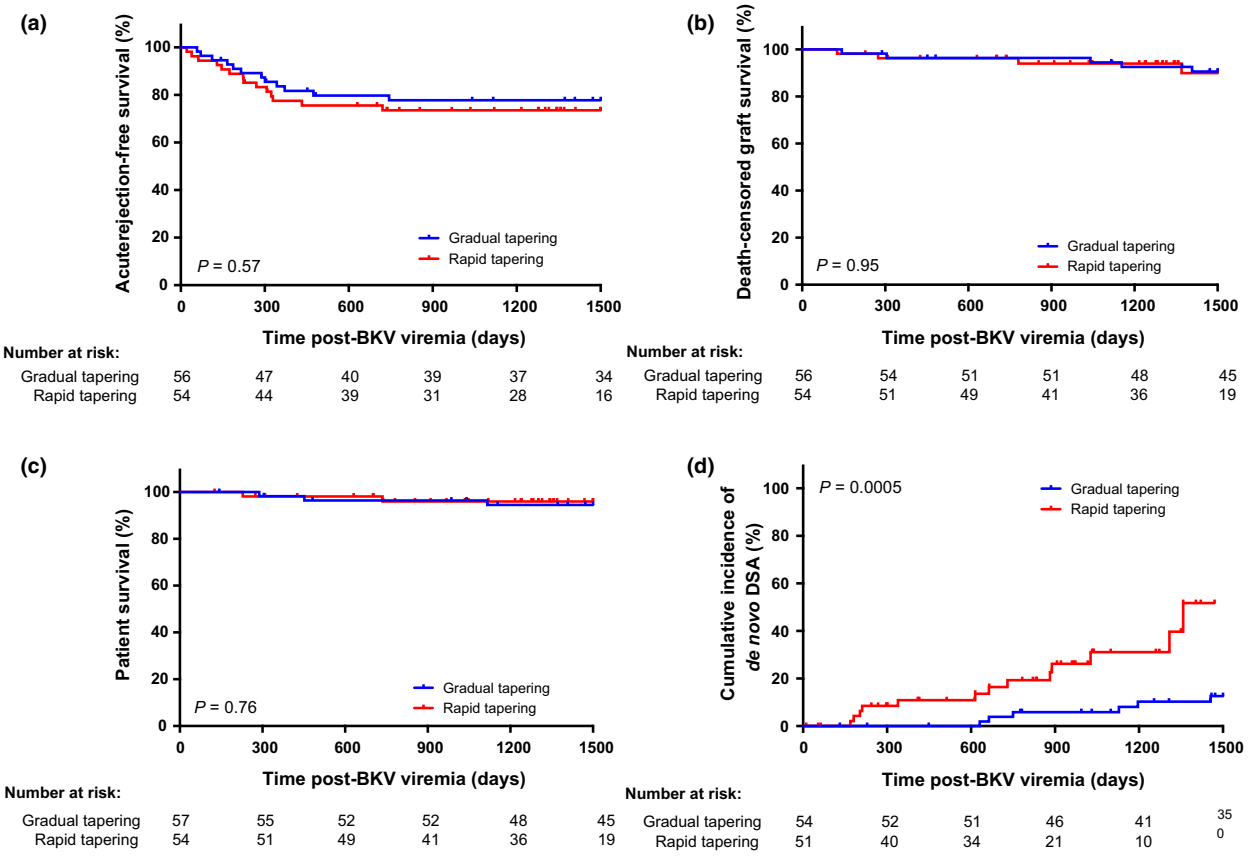

Figure 2 The strategy of immunosuppressive regimen tapering does not affect medium-term patient and allograft outcome but is associated with the development of *de novo* DSAs. Biopsy-proven acute rejection-free survival (a), death-censored graft survival (b), patient survival (c), and the time to *de novo* DSA after the first detectable BKV viremia (d) stratified by the strategy of immunosuppressive regimen minimization are shown (gradual tapering, blue line; rapid tapering, red line). MFI greater than 500 was considered positive. Estimates were obtained using the Kaplan–Meier method and compared using the log-rank test.

The demonstration that a rapid and drastic immunosuppression reduction to treat BKV viremia is an independent risk factor of developing dnDSAs is a concerning problem regarding the well-known negative impact of dnDSAs on long-term allograft survival [23,26,27]. In other settings, minimization of immunosuppression during the initial period after transplantation, including MPA dose reduction [28,29] and Tac dose reduction [30], has been shown to increase the risk of alloimmune response. In the specific context of BKV reactivation, the risk of dnDSAs emergence after drug minimization could be even more severe as it has been shown that the majority of graft infiltrating immune cells during BKVAN are alloreactive cells [31]. In two previously published studies [20,32], the association between the emergence of dnDSAs (mainly class II) and immunosuppression reduction after BKV reactivation was also reported. Interestingly, in our study, if a rapid tapering of immunosuppression was associated with a higher incidence of dnDSAs, it did not impact graft outcomes. Several explanations are possible. First, the intensity of dnDSAs was relatively low, especially in the RT group with a median MFI at 1300. Second, dnDSAs occurred quite long after the onset of BKV viremia, and our follow-up time may have been not long enough to observe their deleterious consequences.

Another key finding of our study is the negative impact of basiliximab induction on the development of dnDSAs. It has been previously reported that basiliximab induction compared to Thymoglobulin® induction was associated with an increased incidence of dnDSAs in moderately sensitized recipients [33]. However, our study is the first, to our knowledge, to demonstrate this association in the setting of immunosuppression minimization in response to BKV viremia.

Our study has limitations. First, unmeasured confounding factors are always possible because of the retrospective comparison of two consecutive cohorts. However, the groups were similar with regard to demographic characteristics (with the exception of donor/recipient age) and baseline immunosuppression. Second, the difference in the

Table 3. Risk factors associated with *de novo* DSA development after BKV viremia (univariate and multivariate Cox models).

Parameters		Hazard ratio (95% CI)	*P* value
Univariate analysis			
Recipient age at transplantation	Per 1-year increment	1.024 (0.992–1.055)	0.134
Recipient sex	Male	1 (–)	
	Female	0.660 (0.282–1.546)	0.339
Retransplantation	No	1 (–)	
	Yes	0.812 (0.320–2.058)	0.661
Donor type	Deceased	1 (–)	
	Living	1.689 (0.664–4.293)	0.271
Donor age	Per 1-year increment	1.005 (0.981–1.029)	0.703
Expanded criteria donor	No	1 (–)	
	Yes	1.469 (0.607–3.554)	0.394
Cold ischemia time	Per 1-h increment	0.994 (0.939–1.053)	0.848
Transplant to BK viremia time interval	Per 1-day increment	0.306 (0.064–1.475)	0.140
Preformed DSA	No	1 (–)	
	Yes	0.790 (0.352–1.771)	0.568
Induction	Thymoglobulin®	1 (–)	
	Basiliximab	2.994 (1.289–6.950)	0.011
Plasma exchanges at induction	No	1 (–)	
	Yes	1.137 (0.486–2.660)	0.766
IVIG at induction	No	1 (–)	
	Yes	0.823 (0.361–1.878)	0.644
Rituximab at induction	No	1 (–)	
	Yes	0.598 (0.204–1.757)	0.350
Delayed graft function	No	1 (–)	
	Yes	0.974 (0.403–2.354)	0.953
Treated BPAR before BK viremia onset	No	1 (–)	
	Yes	2.215 (0.982–4.996)	0.055
Treated BPAR after BK viremia onset	No	1 (–)	
	Yes	0.834 (0.329–2.113)	0.703
Peak BKV viral load	Per 1 $\log_{10}$ copies/ml increment	1.004 (0.702–1.435)	0.984
BK virus-related clinical diagnosis	Sustained viremia	1 (–)	
	Biopsy-proven BKVAN	0.907 (0.375–2.192)	0.829
Immunosuppression minimization strategy	Gradual tapering group	1 (–)	
	Rapid tapering group	5.700 (2.029–16.012)	0.001
Multivariate analysis			
Induction	Thymoglobulin®	1 (–)	
	Basiliximab	3.090 (1.285–7.430)	0.012
Immunosuppression minimization strategy	Gradual tapering group	1 (–)	
	Rapid tapering group	6.021 (1.902–19.067)	0.002
Treated BPAR before BK viremia onset	No	1 (–)	
	Yes	2.386 (0.937–6.078)	0.068
Recipient age at transplantation	Per 1-year increment	1.009 (0.973–1.047)	0.630
Transplant to BK viremia time interval	Per 1-day increment	0.999 (0.997–1.002)	0.654

BKVAN, BK virus-associated nephropathy; CI, confidence interval; DSA, donor-specific antibody; HLA, human leukocyte antigen; IvIg, intravenous immunoglobulin; MFI, mean fluorescence intensity; *n*, number.

screening strategy of BKV reactivation between the two groups could lead to unexpected confounding factors impacting the interpretation of the results. However, the increase in BKV viremia screening after 2012 was because of our wish, at that time, to comply with the more recent recommendations. Third, our results from a single-center study should be replicated. However, our highly phenotyped population with systematic longitudinal screening of BKV viremia, DSAs, and screening biopsies allowed us to thoroughly describe graft and patient outcomes. It is worth noting the high rate of highly sensitized recipients. The important overall immunosuppression received

before viremia and the increased risk of developing antibody response in this population may limit the generalizability of our observations. Moreover, the higher frequency of BKV testing in RT group led to earlier detection and earlier reduction in immunosuppression compared to the GT group. So we cannot exclude that the cumulative immunosuppression prior to reduction in immunosuppression doses may have determined the differential incidence of dnDSAs between groups. However, the frequency of BKV testing in the RT group complies with more recent recommendations. A randomized controlled trial is definitely needed to assess if the current strategy of early diagnosis and rapid immunosuppression minimization is actually long-term effective regarding the fact that patients from the GT group had the same medium-term clinical outcome with less dnDSAs. Fourth, the relatively small sample size cannot exclude a lack of power in assessing long-term graft outcome. Fifth, our study does not provide information about other possible strategies of immunosuppression tapering regarding BKV reactivation (e.g., conversion from Tac to cyclosporine or everolimus). In this setting, mammalian targets of rapamycin inhibitors (mTORi) could be a promising option to prevent or treat BKV reactivation. *In vitro* studies suggest that mTORi could have an antiviral effect [34]. Moreover, recent randomized trials have shown that a combination of reduced doses of calcineurin inhibitor (CNI) with everolimus was associated with a decreased incidence of BKV reactivation after kidney transplantation compared to a combination of standard doses of CNI with MPA [35,36]. The impact of mTORi after BK viremia remains more conflicted. However, a recent retrospective study suggests that a conversion from a standard dose Tac-MPA combination to a low-dose Tac-everolimus combination could be an efficient and safe strategy to rapidly control the infection, even in patients who develop BKVAN [37]. Large-scale, prospective, randomized studies comparing different strategies (including conversion to everolimus-based regimens) are lacking. However, a multicenter, randomized, two-arm study evaluating the BKV viral clearance in kidney transplant recipients with BKV viremia after reduction in immunosuppression alone compared to a reduction in immunosuppression and replacement of MPA by everolimus is currently recruiting in France (ClinicalTrials.gov Identifier: NCT03216967NCT).

The risk of BKV reactivation after kidney transplantation exemplifies the need for more personalized assessment of individual risk, both pre- and post-transplantation. Several pretransplant risk factors of BKV reactivation have been identified [12,13,38]. Emerging evidence suggests that the risk of BKV reactivation may be anticipated at the individual level. For instance, BKV-specific T-cell response [39] and BKV genotype-specific neutralizing antibodies [40] have been suggested to be meaningful predictive markers that allow patient stratification by BKV disease risk before and after transplant. In addition, more sophisticated therapeutic drug monitoring of immunosuppressive drugs may also help in addressing the risk of BKV reactivation. For instance, MPA area under the concentration–time curve (AUC) has been shown to be associated with the risk of BKV reactivation [41].

To conclude, our study demonstrates that, compared to a gradual tapering, an aggressive minimization of immunosuppression is associated with a reduced duration of BKV viremia and a better preservation of graft function in patients with confirmed BKVAN, but results in an increased incidence of dnDSAs. The absence of a clear impact on medium-term renal clinical outcomes suggests that both strategies are acceptable. Large, multicenter, randomized controlled trials are warranted to define the best minimization strategies.

Authorship

DA, AD, and CT designed the study. AD collected the clinical data. AV and MR reviewed the biopsies. DA, AD, CT, LA, AS, JZ, and CL analyzed the data. RS collected and analyzed the anti-HLA antibody data. LM did the statistical analyses. VAF generated the virological data. DA and AD drafted and revised the paper; all authors approved the final version of the manuscript.

Funding sources

None.

Conflict of interest statement

The authors declare no conflict of interest.

Acknowledgements

AD is supported by the National Fund for Scientific Research; DA and CT by the Emmanuel Boussard Foundation, the Day Solvay Foundation and the Centaure Foundation; and AV by the *Société Francophone de Transplantation*.

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