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RESEARCH ARTICLE

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Detection of JCV or BKV viruria and viremia after kidney transplantation is not associated with unfavorable outcomes

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Abstract

Studies analyzing the relationship between BK polyomavirus (BKV) or JC polyomavirus (JCV) infection and kidney transplant (KT) long term clinical outcomes are scarce. Therefore, we evaluated this relationship in a single-center retrospective cohort of 288 KT patients followed for 45.4(27.5; 62.5) months. Detection of BKV viremia in two consecutive analyses led to discontinuation of antimetabolite and initiation of mammalian target of rapamycin inhibitor. Outcome data included de novo BKV and/or JCV viremia and/or viruria after KT, death-censored graft survival and patient survival. BKV viruria and viremia were detected in 42.4% and 22.2% of KT recipients, respectively. BKV viremic patients had higher urinary BKV viral loads at the onset of viruria, when compared to nonviremic patients (7 $\log_{10}$ vs. 4.9 $\log_{10}$ cp/mL, $p < 0.001$). JCV viruria was identified in 38.5% of KT patients; the 5.9% of KT recipients who developed JCV viremia had higher JCV urinary viral loads at the onset of viruria, when compared to non-viremic patients (5.3 vs. 3.7 $\log_{10}$ cp/mL, $p = 0.034$). No differences were found in estimated glomerular filtration rate at the end of follow up, when comparing BKV or JCV viruric or viremic patients with nonviremic patients. No association was found between JCV or BKV viruria or viremia and death/graft failure. Therefore, higher BKV urinary viral loads at the onset could serve as an early maker of over immunosuppression. JCV and BKV replication was not associated with inferior clinical outcomes in KT patients with the above-mentioned immunosuppression strategy.

KEYWORDS
BK virus, JC virus, kidney transplantation

1 | INTRODUCTION

JC polyomavirus (JCV) and BK polyomavirus (BKV) are human polyomaviruses. JCV and BKV cause asymptomatic childhood infection and persist in various sites, including the urinary tract[1] and the central nervous system.[2] Nearly 80% of adults are seropositive for JCV and BKV.[3] Detection of viruria unveils renourinary polyomavirus reactivation.[1,4] Polyomavirus reactivation in the urinary tract occurs in approximately one-third of kidney transplant (KT) patients under the most recent immunosuppression protocols.[5]

In KT recipients, viral replication may lead to polyomavirus-associated nephropathy (PVAN) in 1%–10% of patients with direct

tubular infection and interstitial inflammation, progression to graft fibrosis and graft loss in 15%–38% of patients.[6,7] The leading cause of PVAN is over-immunosuppression associated with emergence of latent BKV which assumes an aggressive behavior in selected individuals.

PVAN has been established as a well-defined entity and is one of the most common viral diseases affecting kidney allograft recipients, with BKV being the most frequent causal agent. The gold-standard approach to prevent PVAN consists in the screening of BKV viremia and prevention of BKV viremia progressing to PVAN through reduction of immunosuppression.[8]

No effective direct antiviral therapy is currently available; thus, since the first case was identified in 1971, reduction of immunosuppression remains the primary strategy for BKV nephropathy.[9]

The relationship between immunosuppression and JCV replication is less well defined than that with BKV replication. JCV-associated nephropathy is a rare complication, and regular monitoring of JCV viruria is not recommended after KT.[10] Furthermore, previous studies showed that either JC viremia or simultaneous urinary reactivation of JCV and BKV after KT are rare phenomena.[11,12] Thus, no clear association have been detected between JCV viruria and inferior outcomes.[12]

Studies analyzing the relationship between BKV and JCV infection and long term clinical outcomes in KT patients are scarce. The present study aims to assess the relationships between BKV or JCV infection with graft and patient survival in a cohort of 288 KT patients followed for a median of almost 4 years.

2 | MATERIAL AND METHODS

2.1 | Study design and population

In this single-center retrospective cohort study, we enrolled 288 consecutive KT patients, who underwent KT between January 2013 and December 2018 at a Kidney Transplant Unit in Portugal, with at least 1 year of follow-up after KT.

JCV and BKV viremia and viruria were evaluated every month for the first 6 months and then every 3 months until 2 years after KT through a commercial real-time quantitative polymerase chain reaction (qPCR) technique. JCV and BKV viremia and viruria continued to be measured quarterly after the second post-KT year, in patients with JCV or BKV viremia detected during the first post-KT year.

2.2 | Data collection

Demographic characteristics (age, gender), type of donation (living/deceased donor), induction and maintenance immunosuppression, immunologic risk profile (number of mismatches between donor and recipient, presence of class I and class II anti-HLA antibodies) were collected at baseline; estimated glomerular filtration rate (eGFR) was evaluated at the end of follow up.

Graft failure was defined as an eGFR < 15 mL/min/1.73 m^2 calculated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation or need to initiate dialysis.[13]

Outcome data included the occurrence of the novo BKV and/or JCV viremia and/or viruria after KT, death-censored graft survival and patient survival.

2.3 | BKV and JCV analysis

BKV viruria and JCV viruria were defined, respectively, by the presence of BKV DNA or JCV DNA in the urine. BKV viremia and JCV viremia were defined, respectively, by detection of BKV virus DNA or JCV virus DNA in plasma.

In both assays, two amplification reactions were performed starting from extracted DNA. For BKV, a specific primer for the region of the Large T antigen gene of BKV and a specific primer for the region of the human beta Globin gene (internal control) were used; for JCV, a specific primer for the large T antigen region of the JCV gene and a specific primer for an artificial sequence of DNA (internal control) were used. BKV- and JCV-specific probes with ELITE MGB® technology, labeled with FAM fluorophore, is activated when it hybridizes with the specific product of the BKV and JCV amplification reaction. Viral load is obtained, in each case, through a calibration curve.

Polyomavirus infections were defined according to recommendations of the Banff working group and the American Society of Transplantation Infectious Diseases Community of Practice guidelines.[8,14] Plasma BKV viral load $\geq 1 \times 10^4$ copies/milliliter (cp/mL) was defined as presumptive polyomavirus nephropathy (pPVAN) and polyomavirus nephropathy (PVAN) was defined by biopsy.

Detection of BKV viremia in two consecutive analyses, led to discontinuation of the antimetabolite and initiation of mammalian target of rapamycin (mTOR) inhibitor (everolimus target 12-h trough levels of 3–7 ng/mL). Calcineurin inhibitor target trough levels were also reduced (tacrolimus target 12-h trough levels of 3–5 ng/mL), in accordance to clinical practice in our center. Prednisolone was kept at 2.5 to 5 mg qday.

2.4 | Immunosuppressive regimen

KT recipients received basiliximab or antithymocyte globulin as induction therapy, except if HLA identical living related donors, in which situation, no induction therapy was used. Basiliximab (20 mg IV) was administered in the 1st and 4th day after KT; antithymocyte globulin (1.25 mg/kg/day IV) was administered since the 1st day and optimally until the 7th day after KT; methylprednisolone (500 mg on 1st day, 250 mg on 2nd day, 125 mg on 3rd, and 80 mg on 4th day IV after KT) was included in all immunosuppressive induction regimens. The choice of the immunosuppressive regimen depended mainly on patient's immunologic profile (% of panel

reactive antibodies; number of HLA mismatches with the donor, preformed donor-specific antibodies). In addition, thymoglobulin induction and delayed introduction of calcineurin inhibitor was prescribed to prevent or treat delayed graft function in the setting of long cold ischemia time or previous ischemic insults to the graft. Initial maintenance immunosuppressive therapy included tacrolimus, mycophenolate mofetil, and prednisone. Tacrolimus was administered orally at 0.15 mg/kg/day divided in two doses and adjusted to maintain a target trough concentration between 4 and 10 ng/mL, depending on the time elapsed after KT. Prednisolone was prescribed since the 5th day after KT (0.6 mg/kg) and was tapered to 5 mg/day during the first 3 months after KT. Mycophenolate mofetil (1000 mg orally twice daily) was started after KT and was reduced if adverse events appeared; it was reduced to 1000–1500 mg daily dose after the first 3–6 months.

2.5 | Kidney transplant biopsies

No surveillance or protocol biopsies were performed. All subjects with a rise in creatinine who received indication biopsies were simultaneously assessed for BKV and JCV viremia at the time of biopsy.

Biopsy-proven acute rejection episodes were classified according to 2019 update of the Banff classification.[15]

Acute cellular rejection (ACR) was treated with methylprednisolone (500 mg/day IV) for 3 days. Antithymocyte globulin was prescribed in steroid-resistant ACR or more aggressive histological cellular rejections. Antibody-mediated rejection (AMR) was treated with a variable combination of plasmapheresis, intravenous immunoglobulin, and/or rituximab.

2.6 | Prophylaxis regimens

All KT recipients received trimethoprim/sulfamethoxazole (480 mg qday) as Pneumocystis jirovecii pneumonia prophylaxis for 1 year. Valgancyclovir (900 mg qday, adjusted to kidney function) was given to patients which induction therapy included antithymocyte globulin and/or rituximab or in receptor CMV Immunoglobulin G (IgG)-negative/donor CMV IgG-positive pairs for 6 months.

2.7 | Statistical analysis

An exploratory analysis was carried out for all variables. Categorical data were presented as frequencies and percentages, and continuous variables as mean (standard deviation) or median and interquartile range (25th percentile; 75th percentile), as appropriate. Comparison of time to first JCV viruria with time to first BKV viruria was performed for patients with only one of the viruses separately from patients with both viruses, using tests of hypotheses for independent and for paired samples, respectively. The same methodology was applied when comparing viral loads. Nonparametric tests (χ^2, Fisher's exact, Mann–Whitney *U*, and Wilcoxon Signed Rank test) were used as appropriate. Additionally, Kaplan–Meier estimator and Cox regression models to analyze time until BKV or JCV diagnosis of infection, patient survival, and graft survival were used.

The level of significance $\alpha = 0.05$ was considered. All data were analyzed using R software (R: A Language and Environment for Statistical Computing, R Core Team, R Foundation for Statistical Computing, Vienna, Austria, 2014).

The study is in compliance with the Declaration of Helsinki, follows national and international guidelines for health data protection and was approved by the Ethics Committee of the "Centro Hospitalar Lisboa Ocidental" (approval number 20170700050).

3 | RESULTS

3.1 | Patients' characteristics

A total of 288 KT patients (115 women; 173 men) with a median age of 52.7 years (42.5; 61.1) were enrolled in this study and followed up for a median time of 45.4 (27.5; 62.5) months after KT. Fifty-three patients (18.4%) received a kidney from a living kidney donor and 47.4% of patients received T-cell depleting as induction therapy. A kidney biopsy was performed in 48 (16.7%) patients. Acute humoral and/or cellular rejection was diagnosed in 26 patients (54.2%) and PVAN in 5 patients (10.4%) who underwent kidney biopsy. Clinical and demographical data at baseline are detailed in Table 1.

3.2 | BKV viruria and viremia

BKV viruria and viremia were detected in 42.4% and 22.2% of KT recipients, respectively. The median time to onset of BKV viruria was 2.6 (1.4–6.1) months and to BKV viremia was 3.3 (2.5–5.1) months. The median of the highest BKV urinary viral load was 7.5 (6.0–8.9) $\log_{10}$ cp/mL and it was 3.6 (2.6–4.8) $\log_{10}$ cp/mL in plasma. BKV viremic patients had higher BKV urinary viral loads at the onset of viruria, when compared to nonviremic patients (7 $\log_{10}$ vs. 4.9 $\log_{10}$ cp/mL, $p < 0.001$).

Characteristics of BKV viruric and BKV viremic patients are described in Tables 2 and 3.

3.3 | JCV viruria and viremia

JCV viruria was identified in 38.5% of KT patients; only 5.9% of KT recipients ever developed JCV viremia. Median time to the onset of JCV viruria was 2.6 (1.6–10.1) months.

JCV viremic patients had higher JCV urinary viral loads at the onset of viruria, when compared to nonviremic patients (5.3 vs. 3.7

TABLE 1 Demographic and clinical characteristics.

Characteristics	Patients (n = 288) Median (P_{25}–P_{75})
Age at KT (years)	52.7 (42.5–61.1)
Time of RRT until KT (months)	59.0 (32.0–87.0)
Cold ischemia time (hours)	17.0 (12.0–20.0)
Time of follow up (months)	45.3 (20.5)[a]
	n (%)
Gender	
Female	115 (39.9)
Male	173 (60.1)
Previous KT	22 (7.6)
Type of donation, deceased	235 (81.6)
Mismatches HLA A/B	
0	11 (3.8)
1	34 (11.8)
2	102 (35.4)
3	93 (32.3)
4	48 (16.7)
Mismatches HLA DR	
0	72 (25.0)
1	133 (46.2)
2	83 (28.8)
Mismatches HLA total	
0	7 (2.4)
1	17 (5.9)
2	44 (15.3)
3	79 (27.4)
4	63 (21.9)
5	54 (18.8)
6	24 (8.3)
Anti HLA class I Ab, positive	142 (49.3)
Anti HLA class II Ab, positive	129 (44.8)
IgG CMV, positive	257 (89.2)
Donor IgG CMV, positive	252 (87.5)
Immunosuppressive therapy	
IMS induction TG	136 (47.4)
IMS maintenance antimetabolite	220 (76.4)
IMS maintenance mTOR inhibitor	72 (25.0)
Graft loss	13 (4.5)
Death, deceased	11 (3.8)
Biopsy	48 (16.7)
Kidney rejection (n = 48)	26/48 (54.2)
Rejection in the first year (n = 48)	16/48 (33.3)
PVAN in kidney biopsy (n = 48)	5 (10.4)
eGFR (mL/min/1.73 m^2)	51.6 (20.4)[a]

Note: eGFR calculated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.

Abbreviations: Ab, antibodies; CMV, cytomegalovirus; HLA, human leukocyte antibodies; KT, kidney transplantation; PVAN, polyomavirus nephropathy; RRT, renal replacement therapy; TG, thymoglobulin.

[a]Mean (SD).

TABLE 2 Characteristics of BKV viruric patients.

Characteristics	Patients (n = 122) Median (P_{25}–P_{75})
Time to 1st BKV viruria (months)	2.6 (1.4–6.1)
Viral load 1st BKV viruria (log_{10}) cp/mL	6.1 (3.6–7.6)
TAC 1st BKV viruria (ng/mL)	8.2 (6.4–10.2)
eGFR 1st BKV viruria (mL/min)	58.0 (42.0–67.3)
Time to higher BKV viruria (months)	4.2 (2.6–9.3)
Viral load higher BKV viruria (log_{10}) cp/mL	7.5 (6.0–8.9)
TAC higher BKV viruria (ng/mL)	7.1 (5.7–8.7)
eGFR higher BKV viruria (mL/min)	57.5 (43.8–68.0)

Abbreviations: BKV, BK polyomavirus; eGFR, estimated glomerular filtration rate; TAC, tacrolimus.

TABLE 3 Characteristics of BKV viremic patients.

Characteristics	Patients (n = 64) Median (P_{25}–P_{75})
Time to 1st BKV viremia (months)	3.3 (2.5–5.1)
Viral load 1st BKV viremia (log_{10}) cp/mL	2.9 (2.4–3.7)
TAC 1st BKV viremia (ng/mL)	7.7 (1.8)[a]
eGFR 1st BKV viremia (mL/min)	56.3 (17.0)[a]
Time to higher BKV viremia (months)	4.0 (2.9–10.2)
Viral load higher BKV viremia (log_{10}) cp/mL	3.6 (2.6–4.8)
TAC higher BKV viremia (ng/mL)	7.0 (1.8)[a]
eGFR higher BKV viremia (mL/min)	52.6 (19.5)[a]

Abbreviations: BKV, BK polyomavirus; eGFR, estimated glomerular filtration rate; TAC, tacrolimus.

[a]Mean (standard deviation).

log_{10} cp/mL, $p = 0.034$). Characteristics of JCV viruric and JCV viremic patients are described in Tables 4 and 5.

No patient expressing JCV viruria or viremia presented progressive multifocal leukoencephalopathy (PML) along the follow up period.

3.4 | Interactions between BKV and JCV

Considering all the patients, no relationship was detected between BKV viruria and JCV viruria, as from the 65 JCV viruric patients, 37.7% were also BKV viruric vs 39.2% that were not ($p = 0.808$).

3.5 | Patients with isolated viruria for BKV or JCV

Still, further analyses were performed regarding times to first viruria and corresponding viral loads, for the 141 patients with only one of the two viruses ($n = 65$ with JCV and $n = 76$ with BKV). The median time to the onset of JCV viruria was 2.0 (1.5–4.4) log_{10} cp/mL and that to the onset of BKV viruria was 2.5 (1.4–4.7) log_{10} cp/mL ($p = 0.749$).

Moreover, the median of the highest JCV viral load in the urine was lower (7.0, 5.4–8.0) log_{10} cp/mL, than the median of highest BKV viral load (7.5, 6.2–8.7 log_{10} cp/mL; $p = 0.046$).

Median JCV urinary viral loads at the onset of viruria (3.8, 2.9–5.4) log_{10} cp/mL were lower when compared to median BKV urinary viral loads at the onset of viruria (6.3, 3.1–7.5) ($p < 0.001$).

TABLE 4 Characteristics of JCV viruric patients.

Characteristics	Patients (n = 111) Median (P_{25}–P_{75})
Time to 1st JCV viruria (months)	2.6 (1.6–10.1)
Viral load 1st JCV viruria (log_{10}) cp/mL	3.9 (3.0–5.9)
TAC 1st JCV viruria (ng/mL)	8.0 (6.2–9.6)
eGFR 1st viruria JC (mL/min)	55.0 (46.0–69.3)
Time to higher JCV viruria (months)	15.6 (9.3–25.0)
Viral load higher JCV viruria (log_{10}) cp/mL	6.9 (5.1–8.0)
TAC higher JCV viruria (ng/mL)	6.7 (5.2–8.1)
eGFR higher JCV viruria (mL/min)	57.2 (18.6)[a]

Abbreviations: BKV, BK polyomavirus; eGFR, estimated glomerular filtration rate; TAC, tacrolimus.
[a]Mean (standard deviation).

TABLE 5 Characteristics of JCV viremic patients.

Characteristics	Patients ($n = 17$) Median (P_{25}–P_{75})
Time to 1st JCV viremia (months)	3.5 (1.9–16.1)
Viral load 1st JCV viremia (log_{10}) cp/mL	3.4 (2.2–5.3)
TAC 1st JCV viremia (ng/mL)	8.8 (6.6–11.3)
eGFR 1st JCV viremia (log_{10}) cp/mL (ml/min)	58.0 (47.5–63.5)
Time to higher JCV viremia (months)	4.0 (2.0–16.8)
Viral load higher JCV viremia (log_{10}) cp/mL	3.7 (2.3–5.3)
TAC higher JCV viremia (ng/mL)	8.0 (5.6–10.6)
eGFR higher JCV viremia (mL/min)	55.8 (18.2)[a]

Abbreviations: BKV, BK polyomavirus; eGFR, estimated glomerular filtration rate; TAC, tacrolimus.
[a]Mean (standard deviation).

3.6 | BKV and JCV coinfection

Simultaneous BKV and JCV viruria was detected in 46 KT patients. For those, the median time to the onset of JCV viruria (4.8, 2.1–15.0) months did not differ from time to the onset of BKV viruria (2.6, 1.5–8.0) months ($p = 0.136$).

Moreover, the median of the highest JCV viral load in the urine was lower, 6.5 (3.9–8.0) log_{10} cp/mL, than the median of highest BKV viral load (7.5, 5.6–9.2; $p = 0.009$).

Median JCV urinary viral loads (4.0, 3.0–6.2) log_{10} cp/mL were lower when compared to median BKV urinary viral loads (5.6, 4.1–8.0) log_{10} cp/mL ($p = 0.002$).

Eight patients developed viremia for both viruses. An association was found between BKV and JCV viremia, as from the JCV viremic patients, 47.1%, also had BKV viremia, and only 20.3% from the JCV nonviremic patients developed BKV viremia ($p = 0.016$).

3.7 | Clinical outcomes

Incidence curves to the onset of BKV viruria and viremia and the onset of JCV viruria and viremia are presented in Figure 1A–D, respectively.

Results of univariable analysis showed that none of patients' baseline characteristics (age, gender, type of donation, previous KT, duration of renal replacement therapy, presence of HLA antibodies before donation, cold ischemia time, induction immunosuppression) were associated with the onset of BKV and/or JCV viruria or viremia. Additionally, no association was found either between the onset of BKV viremia or JCV viremia and acute rejection ($p = 0.101$, $p = 0.458$, respectively). (Supporting Information: Tables 1–4). No episodes of acute rejection were diagnosed after discontinuation of antimetabolite and initiation of mTOR inhibitor.

According to previous results, no multivariable models were obtained.

During follow up, 11 (3.8%) of patients died due to cardiovascular, infectious or neoplastic causes. At the end of follow-up, the death censored graft survival was 87.3% and mean eGFR was 51.6 (SD = 20.4) mL/min/1.73 m^2.

No differences were found in eGFR at the end of follow up, between JCV and BKV viruric and nonviruric patients (54.0, 38.0–70.0 vs. 52.0, 38.5–64.0 mL/min/1.73 m^2; $p = 0.379$, and 52.0,

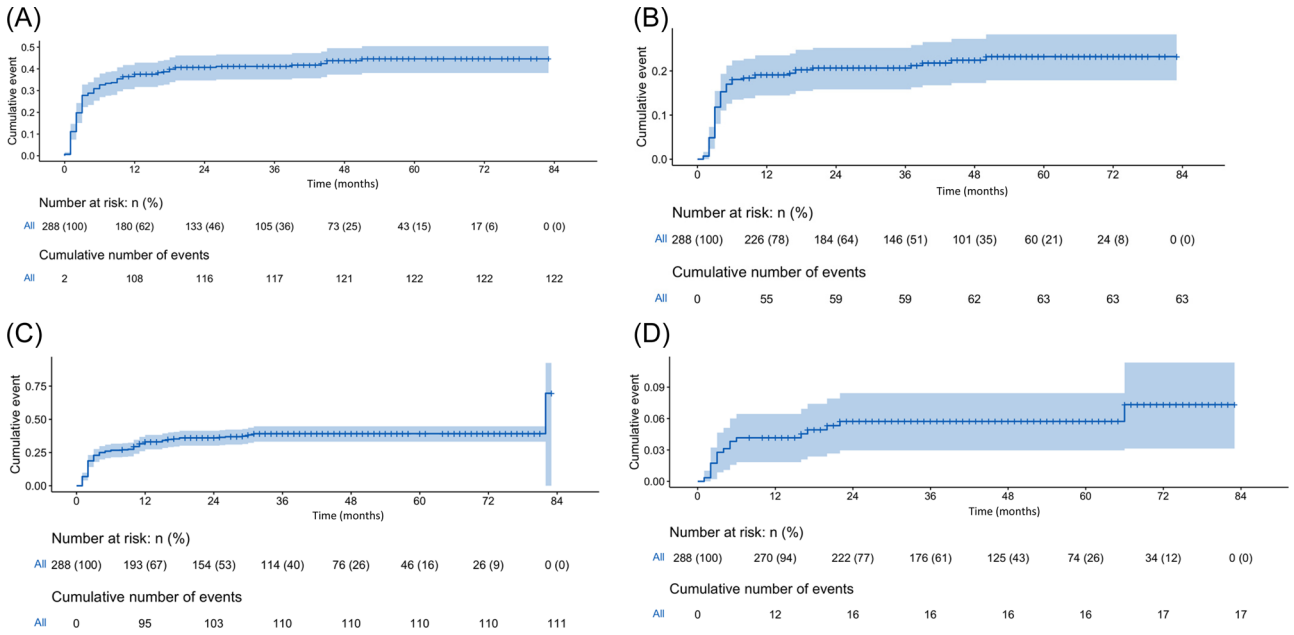

FIGURE 1 (A) Incidence of BKV viruria, (B) incidence of BKV viremia, (C) incidence of JCV viruria, and (D) incidence of JCV viremia.

37.8–63.0 vs. 53, 40.5–67.0 mL/min/1.73 m^2; $p = 0.360$, respectively), as well as between JCV and BKV viremic and nonviremic patients (56.0, 40.0–66.5 vs. 52.0, 38.0–66.0 mL/min/1.73 m^2; $p = 0.658$, and 52.0, 36.0–60.0 vs. 53.0, 39.0–66.0 mL/min/1.73 m^2; $p = 0.537$, respectively).

No association between JCV or BKV viruria and viremia and death/graft failure was found (Supporting Information: Tables 5 and 6).

4 | DISCUSSION

This study represents one of the largest studies evaluating the outcomes of KT recipients expressing JCV or BKV viruria and viremia for a prolonged period.

Although an association was found between BKV viremia and renal damage in selected individuals, there is limited evidence to show an association between BKV viruria or JCV viruria and/or viremia and clinical outcomes. Since a low level of JCV viruria is common in immunocompetent individuals, including in kidney donors,[16] the association between JCV viruria and poor KT outcomes remains controversial. Other than immunosuppression, risks factors for JCV viremia are not known.[17] In previous studies, asymptomatic JCV viruria has been reported in 13.3%–22.6% and viremia was almost undetectable in KT patients.[11,12,18] In this study, we report a much higher incidence of JCV viruria (38.5%) and of JCV viremia (5.9%), although without unfavorable clinical outcomes. Despite the fact that JCV viruria is common in KT patients, only a limited number of PVAN cases have been attributed to JCV.[19,20] Nevertheless, the role of JCV viremia in evaluating the risk of PVAN may be lower as compared to BKV.[21] So, it seems that BKV and JCV have different mechanisms of virus reactivation and shedding.

The relationship between over-immunosuppression and BKV viremia and eventually overt BKV nephropathy is well known.[22] Rates of BKV viruria range between 33% and 35%.[11,12] Additionally, Reischig et al.[23] reported a cumulative incidence of BKV viremia at 3 years posttransplant of 28% and a PVAN incidence of 5%. We report a slightly higher rate of BKV viruria (42.4%) but a similar cumulative incidence of BKV viremia (22.2%).

Additionally, in our cohort no association was found between induction immunosuppression (thymoglobulin vs. basiliximab) and the development of BKV and/or JCV viruria or viremia. Studies regarding the role of immunosuppressive therapy in the development of BKV viremia and nephropathy are conflicting. Nevertheless, more recent publications are in line with our results. Radtke et al.[24] found that neither induction nor maintenance immunosuppressive therapy influenced BKV infection. The same results were recently found by Lorant et al.[25] Authors studied 44 patients with biopsy-proven PVAN and found that enhanced induction (including thymoglobulin), was not associated with BKV PVAN development after KT. We postulated that the use of modern low-dose concepts of immunosuppression in KT, merely impacts polyomavirus replication after KT.

Previous studies tend to show earlier JCV viruria when compared to BKV urinary shedding.[11,12] Conversely, in our study, median time to first detection of JCV viruria was similar to the median time to detect BKV viruria. Additionally, the highest JCV viral load in the urine was lower when compared to the highest BKV viral load. Our data suggest that JCV viruria in KT patients is common, at a lower level compared to BKV excretion, usually being asymptomatic and unremarkable to KT outcomes.

A previous study reported that early BKV infection is a risk factor for BKV viremia and subsequent PVAN.[11] In this study, we showed that the higher the polyoma viral load at the onset of viruria, the greater the risk of developing viremia. This fact is true for both JCV and BKV viruses. This information could be valuable in rehabilitating viruria as a marker for clinical intervention. Recent guidelines suggest stepwise immunosuppression reduction for KT patients with plasma BKV viremia of >1000 copies/mL sustained for 3 weeks or increasing to >10 000 copies/mL.[8] Based on our data, an earlier stepwise immunosuppression reduction for patients with high urinary BKV viral load (above 7 $\log_{10}$ cp/mL) at the onset of viruria might be considered, despite the lack of robust evidence, due to the absence of randomized controlled trials.

Available data on the interactions between JCV and BKV virus remain conflicting. Some authors describe that coinfection with both polyomaviruses is a rare phenomenon, empowering the theory of a possible inhibitory interaction between BKV and JCV in KT patients.[11,12] However, a significant association between JCV viremia and BKV viremia was also seen among our patients. Keyhkhosravi et al.[26] found an association between both viruses in line with the current study. Taken together, these two studies reinforce a mutual support and functional interaction between both viruses. Since both polyomaviruses share overlapping latency sites, the proliferation of one may stimulate the other polyomavirus reactivation. This interaction is only likely to occur in the presence of over-immunosuppression, since the association was verified for viremia and not for isolated viruria.

There is considerable variability of PVAN incidence rates in different transplant centers, which likely reflects differences in the respective programs regarding immunosuppression protocols, polyoma surveillance strategies, as well as biopsy policies for surveillance and indication. We report biopsy-proven PVAN due exclusively to BKV in 1.7% of KT patients. However, none of these patients lost their allograft in follow-up. Some factors could be associated with this phenomenon: first, biopsies were performed only by indication, which could underdiagnose PVAN cases; second, in our immunosuppression protocol, antimetabolite is replaced by an mTOR inhibitor upon detection of BKV viremia in two consecutive analyses. Recent research showed evidence that everolimus reduces the total number of BKV infected cells, which alleviates BKV infection, including nephropathy in KT.[27] Knight et al.[28] sought to determine whether conversion from tacrolimus/mycophenolate mofetil into tacrolimus/mTOR inhibitor immunosuppression would reduce the incidences of BKV and CMV viremia after kidney/pancreas transplantation. Authors found that, 3 years after transplantation, this immunosuppression conversion reduced the incidences of BKV and CMV viremia with an equivalent risk of acute rejection and similar renal/pancreas function. Whether this preemptive immunosuppressive strategy led to the favorable observed outcomes in our cohort is not known. Moreover, we do not report episodes of acute rejection or graft loss associated with this strategy. The challenge in the management of BKV infection is to modulate the balance between the risk of rejection due to under immunosuppression and the risk of graft loss due to PVAN, if immunosuppression is kept at the same level. Thus, further prospective, randomized controlled trials are needed to evaluate the role of the association of mTOR inhibitors with calcineurin inhibitors in minimizing the risk of graft loss due to the competing risks of PVAN progression versus the incidence of acute rejection.

Hertz-Tang et al.[29] followed 1146 KT patients for 35 months and found that incidence of mortality, graft failure, rejections, and infections was not significantly different between BKV viremic and BKV nonviremic patients; however, unlike in our study, JCV was not evaluated. Likewise, we showed that neither BKV viruria nor viremia were associated with inferior graft function, graft survival, patient survival or acute rejection in a long-term follow-up, but we extended the analysis to JCV. Therefore, this study is, to the best of our knowledge, the first to evaluate the impact of JCV on graft and patient survival in a large cohort of KT patients and during a long term follow up.

This was an observational study, with all the biases and limitations inherent in this type of study. Another limitation is that biopsies were only performed for patients with impaired renal function. Therefore, we may have missed subclinical rejection or PVAN.

In conclusion, the present study suggests that JCV and BKV replication was not associated with inferior clinical outcomes in KT patients with the immunosuppression regimens used in this study. Additionally, higher BKV urinary viral loads at the onset of viruria could serve as an early maker of over-immunosuppression, becoming an useful tool to decide on a timely immunosuppression reduction. Conversely, based on our findings, we do not recommend changing immunosuppression based only on JCV viral loads.

Randomized clinical trials would be necessary to validate the role of mTOR inhibitors as a pre-emptive therapy for the prevention of PVAN development in BKV viruric or viremic patients. Although prospective, such trials are unlikely to be performed due to the heterogeneity of KT patients and the long and unpredictable course of PVAN as the cause of renal dysfunction.

AUTHOR CONTRIBUTIONS

Sara Querido: concept/design, data collection, data analysis, drafting article. **André Weigert**: drafting article, critical revision of article. **Iola Pinto** and **Ana Luísa Papoila**: statistics, critical revision of article. **Maria Ana Pessanha** and **Perpétua Gomes**: data analysis/interpretation, critical revision of article. **Teresa Adragão** and **Paulo Paixão**: critical revision of article.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

The study was approved by the Ethics Committee of the "Centro Hospitalar Lisboa Ocidental" (approval number 20170700050).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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