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BK and JC Polyomavirus Infections in Tunisian Renal Transplant Recipients

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The aim of this prospective study was to investigate the rate of BK (BKPyV) and JC (JCPyV) polyomavirus infections and their influence on allograft function in Tunisian renal transplant recipients. A total of 72 renal transplant recipients were studied. BKPyV and JCPyV were detected and quantified by real-time PCR in urine and plasma. Demographic and laboratory characteristics were collected for each patient. Polyomavirus DNAuria was detected in 54 (75%) of renal transplant recipients: 26 (36%) had BKPyV DNAuria, 20 (28%) had JCPyV DNAuria, and 8 (11%) had a dual BKPyV/JCPyV DNAuria. BKPyV DNAemia was detected in four (5.5%) patients, whereas no patient had JCPyV viremia. More than 70% of BKPyV and JCPyV infections started within the first 3 months post-transplant. The risk for positive DNAemia was observed in patients with DNAuria level $>10^7$ copies/ml. BK polyomavirus-associated nephropathy (BKPyVAN) was observed in two patients. This study highlights the high frequency of BKPyV and JCPyV viruria during the first year post-transplant with the highest incidence observed in the third month. We identified several risk factors that were associated with BKV DNAuria including age, sex of patients, and the use of tacrolimus instead of cyclosporine A at month 3. The use of cyclosporine A instead of tacrolimus was identified as risk factor for JCV viruria in month 3. No statistical difference in the allograft function was found between BKPyV and/or JCPyV infected and uninfected patients.

INTRODUCTION

BK and JC polyomaviruses (BKPyV and JCPyV) infects humans commonly. Primary infection with BKPyV and JCPyV usually occurs in early childhood and the seroprevalence rate reaches 65–90% in adults [Knowles, 2001; Hirsch and Steiger, 2003]. After initial infection these viruses may persist lifelong in the kidney epithelium, the genitourinary tract epithelium, and/or lymphocytes [Costa et al., 2009]. BKPyV and JCPyV may be reactivated in immunocompromised individuals such as transplant recipients [Bodorini et al., 2001]. BKPyV is the most common pathogen in renal transplant recipients and BKPyV-associated nephropathy (BKPyVAN) is one of the most significant causes of graft dysfunction and loss in renal transplant recipients [Randhawa et al., 2005]. BKPyVAN may be detected in about 8% of renal allograft recipients with loss of renal allograft ranging from 10% to 80% of cases [Hirsch et al., 2006]. JCPyV could be reactivated with asymptomatic shedding in urine whereas in immunocompromised individuals (mainly AIDS patients), JCPyV reactivation might lead to the progressive multifocal leukoencephalopathy (PMLE) [Kitamura et al., 1990, 1994]. In contrast to the closely related BKPyV, limited information is available currently, regarding the JCPyV. A few cases of nephropathy have been attributed to this virus [Randhawa et al., 2001; Kantarci et al., 2011]. In a recent study, JCPyV associated nephropathy (JCPyVAN) was reported as

KEYWORDS: BK polyomavirus; JC polyomavirus; prospective study; Renal transplant recipients; Tunisia

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low as 0.9% despite the fact that a significant proportion of the patients displayed JCPyV viruria or decoy cells shedding [Drachenberg et al., 2007]. Interestingly, the majority of JCPyVAN was diagnosed in patients with a normal renal function suggesting an apparently less aggressive or more protracted clinical course when compared with BKPyVAN. Other differences were observed between BKPyVAN and JCPyVAN like the strong association with viremia and the severity of histological pattern in the former [Hirsch et al., 2002] and the response to the immunosuppressive regimen that does not play any important role once JCPyVAN is established [Kazory et al., 2003]. The aim of this prospective study was to evaluate the frequency of BKPyV and JCPyV infections in renal transplanted patients, to determine risk factors for virus reactivation and to assess their influence on allograft function during the first year post-transplantation.

MATERIALS AND METHODS

Patients and Samples

A total of 72 adult renal transplant recipients (46 males and 26 females) were included in this study between January 2008 and January 2011. They were recruited from the Kidney Transplantation Departments of Sahloul hospital in Sousse and Fatouma Bourguiba hospital in Monastir. Both hospitals are located in Eastern Tunisia. All patients gave informed consent and the study was approved by the Ethics and Research Committee of the Fatouma Bourguiba hospital. No specific screening was used to include or exclude enrollment. The immunosuppressive regimen consisted of induction and maintenance therapy. The induction was carried out with Anti-thymocyte globulin (ATG) administered from 5 days to 12 days to all patients, except first-time RT recipients. These first-time RT recipients who were not at increased risk for rejection (HLA reactivity against the panel <15% and cold ischemia time <36 hr) and received basiliximab at day 0 and 4. Maintenance therapy consisted of the triple drug regimen: that commonly includes mycophenolate mofetil, prednisone, and cyclosporine A or tacrolimus. Four patients received Rapamune and four others received only mycophenolate mofetil. All the patients were followed up prospectively during the first year post-transplantation. Demographic and clinical data such as sex, age, cold ischemia time, number of HLA mismatches, type of induction and maintenance therapy, and occurrence of rejection were recorded (Table I). Data on CMV, hepatitis B and hepatitis C infections, and levels of creatininemia were recorded at each visit. Urine (30 ml) and peripheral EDTA-blood samples (5 ml) were collected every 2 weeks during the first 3 months and at 6, 9 and 12 months post-transplantation. The number of patients contributing to urine and plasma samples at each sampling

point is presented in Table II. Each patient had between 5 to 12 samples (urine and blood) collected. A total of 586 urines and 616 plasmas samples were collected (Table II). Specimens were stored at 20°C until DNA extraction.

BKPyV and JCPyV Detection

A volume of approximately 30 ml of urine or 5 ml of peripheral EDTA-blood were collected from each patient. DNA was extracted from 0.2 ml of urine and cell-free plasma using the QIAamp DNA mini kit (Qiagen, Courtaboeuf, France) according to the manufacturer's instructions. BKPyV and JCPyV DNA detection was performed using a real-time PCR that allows a simultaneous detection of BKPyV and JCPyV [Whiley et al., 2001]. Briefly, PCR was performed with primers that amplify a common sequence in the VP2 gene, producing a 131 bp product. The probes used, were labeled with a donor fluorescein fluorophore at the 3' end for the first probe, and an acceptor fluorophore, LC-Red 640, at the 5' end. A 3'-phosphorylated residue was used for the second probe. A 20 µl volume was used in each reaction capillary. Capillaries were loaded with a mix containing 5 µl of target DNA, 2 µl of LightCycler FastStart DNA master hybridization probes reagent (Roche Diagnostics, Meylan, France), 2.4 µl of MgCl₂ (25 mM) stock solution, 2 pmol of sense primer, 8 pmol of antisense primer, and 4 pmol of each probe. Each mixture was made up to 20 µl with sterile PCR-grade water. Each run included a positive control and three no-target controls consisting of 15 µl of reaction mixture and 5 µl of PCR-grade water. The PCR conditions using a LightCycler (Roche Diagnostics) apparatus were as follows: 10 min of incubation at 95°C followed by 55 cycles of denaturation at 95°C for 10 sec, annealing at 55°C for 10 sec, and extension at 72°C for 20 sec. The distinction between BKPyV and JCPyV was achieved by melting curve analysis [Whiley et al., 2001].

BKPyV and JCPyV Quantification

A standard curve was generated by amplifying 10-fold serial dilutions (1:10 to 1:10¹⁰ copies) of a PCR amplification product cloned into a vector using the pGEM T Easy PCR cloning system (Promega, Charbonnières, France). The lower limit of detection was 2.0 log copies/ml for both BKPyV and JCPyV. The threshold cycle (CT) was determined as the cycle number at which the sample quantification curve became exponential. Samples with a CT >40 cycles were considered as negative. The limit of 40 Ct is corresponding to about 100 copies/ml according to the standardization of the method.

Statistical Analysis

Statistical analyses were performed with SPSS software for Windows (Statistical Product and Service

TABLE I. Demographic and Clinical Characteristics of the 72 Enrolled Renal Transplant Recipients

Different groups	BK virus infected (n ¼ 26)	JC virus infected (n ¼ 20)	BK and JC virus infected (n ¼ 8)	Uninfected (n ¼ 18)
Sex				
Female no (%)	6 (23.1)	8 (40)	2 (25)	10 (55.6)
Male no (%)	20 (76.9)	12 (60)	6 (75)	8 (44.4)
Age (year)				
Mean SD	24.6 7.5	32.8 7.5	36.2 10.5	34.1 6.5
Median	25	30	39.5	35
Range	15–41	25–50	20–46	21–43
Cadaveric donor_no (%)	0 (0%)	0 (0%)	2 (25)	4 (22.2)
Non cadaveric donor no (%)	26 (100)	20 (100)	6 (75)	14 (77.8)
HLA mismatches_no				
Mean SD	2.7 1.5	2.7 1.8	2.7 1.5	1.8 1.5
Median	3	3	3	1
Range	1–6	0–6	1–6	1–5
Cold ischemia duration_min				
Mean SD	30.7 7.3	31.6 10.25	40 14.1	200 466.9
Median	25	30	40	20
Range	25–40	25–50	30–50	20–1350
Induction therapy_no (%)				
ATG*	20 (76.9)	16 (80)	8 (100)	18 (100)
Basiliximab	6 (23.1)	4 (20)	0 (0)	0 (0)
Maintenance therapy_no (%)				
Prednisone þ MMF þ Cyclosporine	2 (7.7)	6 (30)	0 (0)	2 (11.1)
Prednisone þ MMF þ Tacrolimus	20 (76.9)	10 (50)	8 (100)	16 (88.9)
Prednisone þ MMF þ Rapamune	2 (7.7)	2 (10)	0 (0)	0 (0)
Others	2 (7.7)	2 (10)	0 (0)	0 (0)
Rejection_no (%)	2 (7.7)	2 (7.7)	0 (0)	3 (16.7)
CMV infection_no (%)	6 (23.1)	0 (0)	4 (50)	8 (44.4)
Creatininemia (mmol/L)				
Mean SD (Median)				
3 month	105.5 29.6 (93.5)	108.8 29.6 (107)	109.3 12.07 (109.0)	205 170 (150)
6 month	93.3 20.7 (93.0)	108.5 32.8 (110.5)	118.7 15.5 (113.5)	146.8 73.64 (113.0)
12 month	110.2 30.3 (104.0)	116.0 21.8 (105.0)	112.0 6.7 (113.0)	163.2 119.9 (112.5)

* ATG Anti-Thymocyte Globulin.

Solutions, version 20.0). Quantitative variables were compared using the Kruskal–Wallis test, Student's t-test or Mann–Whitney U test as appropriate, and for qualitative variables differences were evaluated using the chi-square or the Fisher exact test. A multivariate logistic regression model was used to estimate odds ratio (OR) and 95% confidence intervals (CI). All P values were based on a 2-tailed test of significance (P ¼ 0.05).

RESULTS

BKPyV and JCPyV Infections

BKPyV DNA was detected in 82 (14.0%) urine samples of 34 (47.2%) patients, whereas JCPyV DNA

was detected in 56 (9.5%) urine samples of 28 (38.9%) patients. Both viruses were detected in 23 (4%) urine samples of 8 (11.1%) patients. BKPyV DNA was detected in 6 plasmas samples of four (5.5%) patients, whereas JCPyV DNA was not detected in plasma.

Seven patients had a rejection episode confirmed by allograft biopsy. Three patients were negatives for both viruses; one of them lost the graft and subsequently died. Two patients had JCPyV DNA in urine in the first month of transplantation, before the apparition of rejection episode. The two other patients had BKPyV DNA in urine and plasma. BKPyV Viruria was persistent in these two patients from the first to the 12th month, so before and after the diagnosis of rejection. For one of them viremia was

TABLE II. The Number of Patient Contributing to the Urine and Plasma Collection and the Number of Sample at Each Sampling Point

Time after transplantation	1 month	2 month	3 month	6 month	9 month	12 month	Total
No of patients contributing to urine samples	72	69	71	68	70	71	
No of urine samples	131	121	123	68	70	73	586
No of patients contributing to plasma samples	72	70	72	70	71	71	
No of plasma samples	132	133	135	70	73	73	616

present twice before the diagnosis of rejection on the 1st and 3rd month. In contrast for the second patient, the viremia was detected before rejection episode in the 9th month and after in the 12th month, this patient lost the graft with subsequent death. Graft biopsy from these two patients documented atypical BKPyVAN. An isolated increase in serum creatinine was also observed in these two cases. For the first patient, creatinemia passed from 72 to 148 mg / l at the 3rd month and for the second patient it passed from 89 mg / l to 215 mg/l at the 9th month. At the same period, these two patients have presented a hematuria.

Virological Qualitative and Quantitative Data

All the four patients presenting a BKPyV DNAemia had a concomitantly BKPyV viruria with significantly higher viral load in urine (median, 7.8 log copies/ml; range 5.4–9.4 log copies/ml) than that of patients who did not present BKPyV DNAemia (median, 4.3 log copies/ml, range 1.98–7.8 log copies/ml) ($P \leq 0.0001$). The risk of BKPyV DNAemia increased when BKPyV DNA level in urine was greater or equal to 8.3 log copies/ml ($P \leq 0.001$, range 8.3–9.4 log copies/ml). Patients presenting DNAuria during the course of the follow-up were at risk for DNAemia. Indeed, all the four patients presenting BKPyV DNAemia had at least six episodes (range, 6–12 episodes) of BKPyV viruria, simultaneously before and after the viremia appearance. Whereas patients with no BKV DNAemia presented a significantly lower number of BKPyV viruria (range, 1–5 episodes) ($P \leq 0.001$). No patients with JCPyV DNAuria presented JCPyV DNAemia.

BKPyV and JCPyV Infection Kinetics in Patients

Samples were collected over a period of 1–12 months post-transplantation. The number of evaluated patients at each sampling point and related PCR results are presented in Table III. The kinetics of viral excretion was analyzed in all patients of the two groups of BKPyV and JCPyV infected patients. In the BKPyV group, DNAuria occurred in 28 (82%) patients within the first 3 months post-transplantation, the highest viral load (median 7.8 log DNA copies/ml) being observed in the third month. Fourteen patients showed only one positive urine sample and eight patients showed two positive urine sample during the follow-up. These 22 patients, had BKPyV DNA levels in urine (median 3.7, range, 1.9–7.3) lower than those observed among the twelve patients who tested positive more than twice (median 6.9, range 4–9.4, $P \leq 0.01$). In the JCPyV group, DNAuria occurred in 71% of patients within the first 3 months post-transplantation. Eighteen patients showed one positive urine sample during the follow-up, the other 10 patients were tested positive more than once. Unlike BKPyV infection, there was no significant difference in the JCPyV DNAuria level between

patients presenting one positive urine sample or more ($P = 0.754$). The first occurrence of BKV DNAemia was observed within the first 3 months post-transplantation in three of four patients (75%). Two patients tested positive only once for DNAemia (2.5, 2.7 log copies/ml) and remained positive for DNAuria. The two other patients were positive twice for DNAemia, one was positive at 1 and 3 months (2.9 and 4.7 log copies/ml, respectively) and the other at 3 and 9 months post-transplantation (5.2 and 4.6 log copies/ml, respectively). DNAemia disappeared before DNAuria in three patients and remained positive until month 12 in one patient.

Risk Factors Analysis

When considering each group of patients separately, no significant association was found between BKPyV or JCPyV infection and i) number of HLA mismatches, ii) induction therapy, iii) cold ischemia, iv) acute rejection, v) type of donor or vi) active infection with CMV. Serum creatinine levels did not significantly differ among patients from all four groups and no correlation was found between these parameters and viral DNA levels in urine and plasma. By univariate analysis patients infected by BKPyV were younger than the non-infected patients (median, 25 years vs. 34 years, $P < 0.0001$), multivariate analysis confirmed these findings ($P \leq 0.001$; OR 1.32; 95%CI 1.09–1.39). Other potential determinants of BKV replication were associated with BKPyV DNAuria, a trend toward a higher rate of viruria for male patients was found (male, 43.5%; female, 23.1%) ($P \leq 0.004$; OR 18.62; 95%CI, 2.59–133.80) and higher rates of BKPyV DNAuria at month 3 with tacrolimus treatment as compared to cyclosporine A treatment ($P \leq 0.031$; OR, 22.03; 95%CI, 1.62–299.76). For the JCPyV, cyclosporine A treatment was associated with higher rate of JCPyV viruria at month 3 than tacrolimus treatment (60% vs. 18.5%, $P \leq 0.005$), this result was also confirmed by multivariate analysis ($P \leq 0.015$; OR 0.28; 95%CI 0.08–0.667).

DISCUSSION

This is, the first prospective study in Tunisia on BKPyV and JCPyV infection, including viral DNA detection in both urine and plasma, in renal transplant recipients. The overall prevalence of 75% observed for polyomavirus DNAuria is consistent with the 14–77% prevalence range reported by previous studies [Priftakis et al., 2000; Leung et al., 2002; Helantera et al., 2009; Hu et al., 2011; Chehadeh et al., 2013; Delbue et al., 2013]. The incidence rates of 36% and 28% observed for BKPyV DNAuria and JCPyV DNAuria, respectively are also in agreement with previous studies reporting prevalence rates ranging from 23% to 57% for BKPyV DNAuria and 3–27% for JCPyV DNAuria [Hirsch and Steiger, 2003; Knowles, 2006; Drachenberger et al., 2007;

TABLE III. Frequency of BKPyV and JCPyV Infection During the First Year Post Renal Transplantation

Time after transplantation	1 month	3 month	6 month	9 month	12 month
No of new patients (%) with BKPyV DNAuria each month	20 (59)	8 (23)	3 (9)	1 (3)	2 (6)
Total number of patients with BKPyV DNAuria each month	20	23	12	5	6
Median BKPyV DNAuria (Range on log ₁₀ copies/ml)	3.5 (1.9–6.9)	7.8 (3.4–9.4)	5.9 (4–9.4)	4.9 (1.9–7.8)	4.4 (1.9–6.9)
No of patients with BKPyV DNAemia	1	3	0	1	1
No of new patients (%) with JCPyV DNAuria each month	14 (50)	6 (21)	6 (21)	2 (7)	0 (0)
Total number of patients with JCPyV DNAuria each month	14	13	8	3	0
Median JCPyV DNAuria (Range on log ₁₀ copies/ml)	3.3 (3.1–5.6)	5.6 (4.4–6.9)	4.6 (4.3–7.1)	3.2 (2.9–4.4)	0

Helanterä et al., 2009; Pires et al., 2011]. In the present study, a dual BKPyV and JCPyV DNAuria was observed in 11% of patients in agreement with the report of Drachenberg et al. [2007]. Lower frequencies (1.5–3.5%) of dual BKPyV/JCPyV DNAuria have been reported by others [Priftakis et al., 2000; Cheng et al., 2011; Taheri et al., 2011; Kusne et al., 2012], and this low frequency of coinfection was explained by an inhibitory interaction between the two viruses [Cheng et al., 2011].

In the present study BKPyV DNAemia was observed for 5.5% of patients and JCPyV DNAemia was not observed. This BKPyV DNAemia incidence is lower than the 13–29% incidence rates reported by others [Hirsch et al., 2002; Koukoulaki et al., 2009; Sood et al., 2012] but is in agreement with the 7% incidence reported in a recent longitudinal study. [Kusne et al., 2012]. The absence of the JCPyV DNAemia is not surprising since it is known that JCPyV DNAemia is uncommon and most often asymptomatic [Randhawa et al., 2005; Drachenberg et al., 2007; Helanterä et al., 2009; Cheng et al., 2011].

As reported by many studies, most of BKPyV and JCPyV infections (> or equal to 50%) occurred within the first three months after transplantation, these infections are less frequently at the end of the first year and during the two and three years post-transplant [Gardner et al., 1984; Bressollette-Bodin et al., 2005; Huang et al., 2010; Thakur et al., 2011]. The peak incidence of BKV replication appears to be at 6 months in the study of Hirsch et al. and Dadhania et al. This may be due to the differences in the intensity of immunosuppression related to the therapeutic protocol. In addition, Hirsch et al. reported that DNAemia was detected at a median of 23 weeks after transplantation, this may be explained by the fact that their follow-up started at 3 months post-transplantation and not at the first month [Hirsch et al., 2002; Dadhania et al., 2008]. Patients testing positive for BKV DNAuria during the course of the follow-up were at risk for BKV DNAemia which was associated with high BKV DNAuria levels. This observation might confirm the hypothesis that the

BKPyV viremia corresponds to the reactivation of BKPyV in the renal tubular epithelium, leading to the passage of the virus into the bloodstream through the peritubular capillary system [Nickeleit et al., 2000]. In addition, approximately 30–50% of kidney transplant patients with high-level viruria progress to BKV viremia and histologically and clinically manifest PyVAN [Nickeleit et al., 2000; Drachenberg et al., 2007; Viscount et al., 2007].

A BKPyV associated nephropathy occurred in 2 (3%) patients; this frequency is consistent with that reported in the literature [Hirsch et al., 2002; Randhawa et al., 2004; Drachenberg et al., 2007]. These two patients reached the cut-off BKV DNAemia value of 10⁴ copies/ml proposed to be presumptive of BKPyV-associated nephropathy [Hirsch et al., 2002; Randhawa et al., 2004; Hirsch et al., 2006; Drachenberg et al., 2007]. However the other two patients without BKVAN had blood viral loads below the threshold.

In the present study, it was observed that younger age, male subjects and tacrolimus therapy at 3 months post-transplantation increased the risk of BKPyV DNAuria, and that the risk of JCPyV DNAuria increased under cyclosporine A at 3 months. Several studies have reported that the tacrolimus and mycophenolate mofetil combination increases significantly the risk of BKPyV infection and BKPyV-associated nephropathy compared to the cyclosporine A/mycophenolate mofetil combination [Mengel et al., 2003; Brennan et al. 2005; Hirsch et al., 2013]. Mengel et al. showed that the tacrolimus/mycophenolate mofetil/steroids combination increases 8 times the BKPyV incidence and 13 times the risk of BKPyV-associated nephropathy compared to negative polyomavirus subjects [Mengel et al., 2003].

Other studies found that corticosteroids enhanced the risk for BKV replication, and the early withdrawal of corticosteroids protocol was associated with a reduced risk of BKV replication [Pavakis et al., 2005; Dadhania et al., 2008]. In our study all recipients were managed with the steroid maintenance regimen. We need further investigation using

a randomized controlled trial design comparing steroid maintenance versus early steroid withdrawal. On the other hand, Hirsch et al. found that the role of steroids, was not associated with BKV viruria, but BKV viremia was significantly associated with a higher cumulative steroid exposure [Hirsch et al., 2013]. So it's not surprising that we did not find any association with cumulative steroid impact as in our series viremia was found only in four patients. Anti-rejection treatment corticosteroid bolus was also identified as a risk factor for the enhancement of BKV pathogenicity [Hirsch et al., 2002; Randhawa et al., 2004]. The low incidence of rejection at our institution (only seven patients from 72) may partly account for the lack of this association in our series. Another risk factor like ATG-induction treatment was largely discussed and results were controversial. In induction therapy anti-lymphocyte preparations could not be identified as an independent risk factor for BKV viruria, viremia or BKVN [Hirsch et al., 2002; Buehrig et al., 2003; Brennan et al., 2005; Wong et al., 2006]. This is in accordance with our results. Looking at the role of anti-lymphocyte preparations in the treatment of rejection their use can be associated with BK virus replication [Binet et al., 1999; Hirsch et al., 2002]. In their analysis of HLA mismatching and risk of BK virus nephropathy Awadalla et al. showed that BK virus nephritis is associated with a greater number of rejection episodes and a higher incidence of steroid-resistant rejection requiring anti-lymphocyte treatment [Awadalla et al., 2004]. In our study all the patients were treated with corticosteroid bolus so the impact of ATG can't be interpreted in our cohort.

In the other hand, younger age and male subjects were identified as independent risk factors for BKPyV viruria. Male subjects have been reported as a risk factor for BKPyV-associated nephropathy in some single-center studies [Ramos et al., 2002; Khamash et al., 2007]. However, unlike the results reported here, older aged subjects have been reported as being associated with BKPyV infection [Ramos et al., 2002; Khamash et al., 2007; Hirsch et al., 2013], this might be due to the young age of the patients included in the present study (median 34 years).

However, most studies have failed to find a correlation between the frequency of JCPyV viruria and the use of immunosuppressive drugs [Koralnik et al., 1999; Randhawa et al., 2005]. In agreement with the present results, a recent study has demonstrated that cyclosporine A is a risk factor for both JCPyV and CMV infections [Hu et al., 2011].

It was not observed a difference in plasma creatinine levels between the BKPyV or JCPyV-infected and uninfected patients, except some transient increases of creatinine levels in some patients and in the two patients with BKPyVAN. This is consistent with previous reports [Merlino et al., 2004; Helanterä et al., 2009]. However, other studies found the opposite, but those studies included patients from

1 day to more than 25 years post-transplantation [Muñoz et al., 2005; Doucette et al., 2008].

One limitation of the present study was the small number of enrolled patients, this explain the wide confidence intervals found sometimes with the risk factor analysis. So a long-term study with enough sample size is needed to have more information about the role of polyomaviruses on graft function in kidney transplant recipients. As, BKPyV-associated nephropathy has been reported to occur from 2 months to 60 months post-transplantation [Trofe et al., 2004], the 12-month followed is a short period to reveal enough cases of BKPyV-associated nephropathy and/or graft dysfunction. So, according to this and to other studies, a follow-up during 24 months post-transplantation seems to be reasonable [Brennan et al., 2005; Hirsch et al., 2005; Hariharan, 2006].

In summary, results from this study highlight the high frequency of BKPyV and JCPyV viruria within the first 3 months after renal transplantation. This study confirms also the uncommon incidence of JCPyV viremia and nephropathy in the renal transplant population. On the other side, tacrolimus is associated with a higher risk than cyclosporine A regarding BKPyV DNAuria at months 3 and, conversely, cyclosporine A is associated with a higher risk than tacrolimus for JCPyV DNAuria. In addition, male subjects and younger age appear as independent risk factors for BKPyV DNAuria. Together, these data suggests that there are different patterns of BKPyV and JCPyV reactivation.

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