Risk Factors for BK Virus Infection in the Era of Therapeutic Drug Monitoring

Claire Borni-Duval, Sophie Caillard, Jérôme Olagne, Peggy Perrin, Laura Braun-Parvez, Françoise Heibel, and Bruno Moulin

Background. Overimmunosuppression is a widely recognized risk factor for BK virus (BKV) infection, particularly with the combination of tacrolimus, mycophenolate mofetil (MMF), and steroids. Nevertheless, the exact impact of exposure to tacrolimus and MMF is not well understood.
Methods. We examined 240 kidney recipients between 2006 and 2008. BKV was monitored every 2 months in the urine or blood. A kidney biopsy was performed when viremia exceeded 10^4 copies/mL.
Results. Ninety-five (40%) patients had sustained viruria, 48 (20%) sustained viremia, and 17 (7%) biopsy-proven polyomavirus-associated nephropathy. The mean time-to-occurrence was 7.6, 7.9, and 9.7 months for viruria, viremia, and polyomavirus-associated nephropathy. Risk factors associated with BKV infection in univariate analyses were retransplantation, panel-reactive antibody more than 0%, cytomegalovirus D+/R-, cold ischemia time, delayed graft function, induction with antithymocyte globulins, acute rejection before month 3 (M3), tacrolimus trough levels more than 10 ng/mL, and M3 $AUC_{0-12\ hr}$ more than 50 hr mg/L. Multivariate analyses showed that cytomegalovirus D+/R- (adjusted hazard ratio [AHR], 2.03; P=0.05), acute rejection (AHR, 5.4; P<0.001), and mycophenolic acid $AUC_{0-12\ hr}$ more than 50 hr mg/L (AHR, 3.6; P=0.001) were risk factors for BKV.
Conclusions. This study identified a link between a state of increased immunosuppression and BKV infection, especially in patients with higher MMF exposure and elevated tacrolimus trough levels at M3.

Keywords: BK virus nephropathy, Therapeutic drug monitoring, Kidney transplantation, Mycophenolate mofetil, Tacrolimus.

(*Transplantation* 2013;95: 1498–1505)

Polyomavirus-associated nephropathy (PyVAN) has been known to be a cause of graft dysfunction in kidney transplantation since the late 1990s (*1–4*). In previous series, 30% to 50% of kidney recipients developed viruria in the first 3 months (*5, 6*), 10% to 15% progress to viremia, and 1% to 10% have PyVAN (*7*) that leads to graft loss in 10% to 80% of cases (*8*). In the absence of documented antiviral treatment, early diagnosis is essential. Detection of BK virus (BKV) DNA in plasma by polymerase chain reaction (PCR) at a level above 10,000 copies/mL is associated with a high risk of PyVAN ("presumptive PyVAN"), with a positive predictive value of 75% and sensitivity of 97% (*9, 10*), and justifies intervention (reducing immunosuppression) and a graft biopsy. Histology remains the gold standard to confirm the diagnosis. Despite many studies, the risk factors for PyVAN remain unclear. Potential patient determinants include being male, age more than 50 years (*11*), and low immunity against BKV when the donor has a high level of antibodies against BKV (*12*). Negative serostatus of the recipient before transplantation is a controversial risk factor (*12–14*). Ischemia-reperfusion lesions appear to facilitate BKV reactivation (*15*). Human leukocyte antigen (HLA) mismatches (*16*) and a lack of HLA-C7 (*12*) have been described as risk factors in some but not all studies (*5, 11*). The intensity of overall immunosuppression is the only widely accepted risk factor. The triple combination of tacrolimus, mycophenolate mofetil (MMF), and steroids (*8, 9, 17, 18*) has been suggested to play a major role in the development of PyVAN. Nevertheless, PyVAN has been described with other immunosuppressive drugs (*19*), including cyclosporine A (CsA), azathioprine (*5*), and sirolimus (*20–22*). Antilymphocyte preparations could have an impact on the occurrence of BKV infection, but the data are controversial (*13, 23, 24*). Previous episodes of acute rejection and antirejection treatment, including steroid pulses, have been associated with BKV replication and PyVAN (*13, 16, 17*). Despite the recognized role of tacrolimus and MMF in BKV

The authors declare no funding or conflicts of interest.
Nephrology-Transplantation Department, University Hospital, Strasbourg, France.
Address correspondence to: Dr. Claire Borni-Duval, Service de Néphrologie, Nouvel Hôpital Civil, 1, Place de l'Hôpital, BP 426, 67 091 Strasbourg Cedex, France.
C.B.-D. and S.C. participated in the research design, writing of the article, performance of the research, and data analysis. J.O., P.P., L.B.-P., and F.H. participated in the performance of the research. B.M. participated in the research design.
E-mail: claire.borniduval@chru-strasbourg.fr
Received 10 January 2013. Revision requested 29 January 2013.
Accepted 13 March 2013.

ISSN: 0041-1337/13/9512-1498
DOI: 10.1097/TP.0b013e3182921995

infection, the impact of exposure to these drugs is not yet clear. Tacrolimus trough levels more than 8 ng/mL were significantly associated with PyVAN (*17*), and a reduction of trough levels from more than 9 to 6 ng/mL together with tapering MMF resulted in the improvement of PyVAN (*25*). No association was found between MMF doses and the risk for PyVAN (*26*), and no study has evaluated patient risk by measuring the mycophenolic acid area under the curve (MPA AUC). Uniformly accepted threshold values for these drug levels have not yet been identified (*11, 13, 16*).

The purpose of this study was to identify risk factors for PyVAN in a series of 240 kidney transplant recipients and to study the effect of the two major drugs involved in BKV infection by considering patients' drug exposure to tacrolimus and MMF.

RESULTS

Characteristics of Patients

A total of 240 patients were included in our study, 153 (64%) men and 87 (36%) women, with a mean age of 47.1±13.7 years (range, 18–75 years). The primary kidney diseases were 99 (41%) glomerulonephritis, 41 (17%) tubulointerstitial chronic nephropathy, 25 (10%) diabetic nephropathy, 36 (15%) autosomal polycystic kidney disease, 19 (8%) vascular nephropathy, and 20 (8%) other nephropathy or undefined. The characteristics of the patients are detailed in Table 1. The mean cold ischemia time (CIT) was 16.7±7.4 hr (17.8±6.4 when living donors were excluded). The mean donor age was 47.3±15.9 years (range, 14–79 years). At discharge, 59% of patients received CsA and 41% received tacrolimus. Nearly all of the patients were treated with MMF (96.6%) and steroids (97.5%). At month 3 (M3), 129 (54%) patients received CsA, 106 (44%) received tacrolimus, and 5 (2%) were treated with sirolimus. A total of 222 (92.5%) patients were treated with MMF, 10 (4.2%) with MPA, and 8 (3.3%) with everolimus. The mean M3 MPA AUCs for patients treated with tacrolimus and CsA were 41.1±16 and 32.5±15 ng/mL, respectively. A total of 150 (62.5%) patients remained on steroids at 3 months. A total of 127 (53%) patients had a cytomegalovirus (CMV) infection, and 47 (20%) displayed Epstein-Barr virus (EBV) replication. During the follow-up, 12 patients died with functioning grafts, whereas 14 subjects lost their graft. For the remaining patients, the follow-up ranged between 24 and 60 months (median, 38 months).

Description of BK Infection

During the study period, 116 (48%) patients had BKV, 95 (39%) had sustained BKV, and 65 (27%) had a high-level viruria. Sixty (25%) patients had BKV, 48 (20%) had sustained viremia, and 17 (7%) had proven-biopsy PyVAN. The mean (range) occurrence time was 7.6±7.2 (0–41) months after transplantation for viruria, 7.9±8.9 (0–46) months for viremia, and 9.7±7.3 (3–27) months for PyVAN. Cumulative incidences of different types of BKV infection are shown in Figure 1. The characteristics of patients according to the type of BK infection are given in Table 1. Of the 40 patients who presented high-level viremia, 20 had graft dysfunction and 19 were biopsied (one patient denied consent to biopsy). PyVAN was diagnosed in 11 subjects. Of the remaining 20 patients without graft dysfunction, 13 with sustained viremia were biopsied and 6 displayed PyVAN.

At the time of PyVAN diagnosis, 13 patients were treated with tacrolimus, 4 with CsA, 15 with MMF or MPA, 2 with everolimus, and 12 with steroids. Two of the 14 cases who lost their graft (occurring at 12.8 and 26.9 months after transplantation, respectively) were due to PyVAN.

Risk Factors for Sustained Viremia and PyVAN

The area under the receiver operating characteristic (ROC) curve of M3 MPA AUC for the prediction of impending PyVAN was 0.76 (P=0.002). The cutoff value of 50 hr mg/L had a sensitivity of 46% and a specificity of 83% for predicting PyVAN. Twenty-two of the 106 (21%) patients treated with tacrolimus and 14 of the 129 (11%) patients treated with CsA had a MPA AUC more than 50 hr mg/L at M3. Similar results were obtained when the ROC curves of patients treated with CsA+MMF (AUC=0.97; P=0.024) or tacrolimus+MMF (AUC=0.71; P=0.025) were analyzed separately. We found no relevant association between sustained viremia, PyVAN and recipient age, gender, primary kidney disease, donor characteristics such as age, cause of death, extended-criteria donor (ECD), deceased- or living-donor EBV infection in the first 3 months, or treatment with oral steroids at M3. Conversely, retransplantation, panel-reactive antibody (PRA) more than 0%, CIT 20 hr or more, delayed graft function (DGF), CMV mismatch, induction therapy with antithymocyte globulins (ATG; P=0.05 and 0.26), and acute rejection during the first 3 months were risk factors for sustained viremia and PyVAN (Figs. 2 and 3). Body mass index more than 30 kg/m^2 was associated with a higher incidence of sustained viremia.

In terms of maintenance immunosuppression, there was no association of tacrolimus, CsA, and MMF doses with BKV sustained viremia and PyVAN. Treatment with tacrolimus (compared with CsA or proliferation signal inhibitor) was associated with a higher incidence of sustained viremia (P=0.006) and PyVAN (P=0.02). Among patients who received ATG as induction treatment, recipients with tacrolimus (n=95) had a greater likelihood of developing sustained viremia (P=0.005) and PyVAN (P=0.005) than those treated with CsA (n=89). Patients with tacrolimus trough levels more than 10 ng/mL at M3 had a higher incidence of sustained viremia (P=0.0017) and PyVAN (P=0.0006). Higher exposure to CsA (trough level at M3 > 160 ng/mL) was not linked with a higher risk of BK infection (no PyVAN in patients with CsA trough level >160 ng/mL and three cases in patients with CsA trough level <160 ng/mL). Higher exposure to MMF at M3 (>50 hr mg/L) was associated with sustained viremia (P<0.0001) and PyVAN (P=0.013). Patients treated with a combination of tacrolimus and MMF had a higher incidence of sustained viremia and PyVAN (P=0.005 and 0.014, respectively), especially when the M3 MPA $AUC_{0–12\ hr}$ was higher than 50 hr mg/L and the M3 tacrolimus trough level was higher than 10 ng/mL (P<0.0001 and P=0.0004; Figs. 2B and 3B).

In the multivariate analysis (Table 2), CMV mismatch and acute rejection during the first 3 months were associated with sustained viremia and PyVAN. A M3 MPA AUC of more than 50 hr mg/L were highly associated with sustained viremia (adjusted hazard ratio [AHR], 3.6; P<0.001) and PyVAN

TABLE 1. Patient characteristics according to type of BKV replication or infection

	No BKV	Viruria	Sustained viruria	High-level viruria	Viremia	Sustained viremia	PyVAN	Total
Number of patients	124	116	95	65	60	48	17	240
Male gender, n (%)	79 (63.7)	74 (63.8)	56 (58.9)	36 (55.4)	36 (60)	27 (56.2)	9 (52.9)	153 (63.7)
BMI>30 kg/m^2, n (%)	8 (6.5)	12 (10.3)	11 (11.6)	11 (16.9)	9 (15)	8 (16.7)	1 (5.9)	20 (8.3)
Deceased donor, n (%)	114 (91.9)	109 (94)	90 (94.7)	63 (96.9)	58 (96.7)	47 (97.9)	17 (100)	223 (92.9)
ECD, n (%)	42 (33.9)	30 (25.9)	22 (23.2)	14 (21.5)	14 (23.3)	10 (20.1)	4 (23.5)	72 (30)
Graft rank, n (%)								
First	99 (79.8)	72 (62.1)	56 (58.9)	36 (55.4)	36 (60)	27 (56.2)	9 (52.9)	171 (71.2)
Second	24 (19.4)	40 (34.5)	35 (36.8)	26 (40)	22 (36.7)	19 (39.6)	6 (35.3)	64 (26.7)
Third	1 (0.8)	4 (3.4)	4 (4.2)	3 (4.6)	2 (3.3)	2 (4.2)	2 (11.8)	5 (2.1)
PRA > 0%, n (%)	33 (26.6)	41 (35.3)	37 (38.9)	29 (44.6)	24 (20.7)	22 (45.8)	10 (58.8)	74 (30.8)
DGF, n (%)	24 (19.4)	28 (24.1)	25 (26.3)	20 (30.8)	18 (30)	15 (31.2)	6 (35.3)	49 (20.4)
Induction agent, n (%)								
Thymoglobulin	92 (74.2)	94 (81)	80 (84.2)	56 (86.2)	53 (88.3)	43 (89.6)	15 (88.2)	186 (77.5)
Anti-IL-2R	32 (25.8)	22 (19)	15 (15.8)	9 (13.8)	7 (11.7)	5 (10.4)	2 (11.8)	54 (22.5)
ACR in the first 3 months, n (%)	4 (3.2)	13 (11.2)	11 (11.6)	8 (12.3)	10 (16.7)	9 (18.7)	4 (23.5)	17 (7.1)
M3 CNI, n (%)								
CsA	80 (64.5)	49 (42.2)	41 (43.2)	23 (35.4)	24 (40)	15 (31.2)	4 (23.5)	129 (53.7)
Tacrolimus	43 (34.7)	63 (54.3)	51 (53.7)	40 (61.5)	35 (58.3)	32 (66.7)	13 (76.5)	106 (44.2)
None	1 (0.8)	4 (3.5)	3 (3.1)	2 (3.1)	1 (1.7)	1 (2.1)	0 (0)	5 (2.1)
M3 tacrolimus								
T0 level>10 ng/mL, n (%)	21 (16.9)	30 (25.9)	25 (26.3)	21 (32.3)	18 (30)	17 (35.4)	9 (52.3)	51 (21.2)
M3 MMF treatment, n (%)	116 (93.5)	106 (91.4)	87 (91.6)	57 (87.7)	54 (90)	43 (89.6)	14 (82.4)	222 (92.5)
M3 MPA AUC>50 hr mg/L, n (%)	15 (12.1)	23 (19.8)	19 (20)	17 (26.2)	16 (26.7)	16 (33.3)	7 (41.2)	39 (16.2)
M3 treatment: tacrolimus+MMF	43 (34.7)	63 (54.3)	51 (53.7)	40 (61.5)	35 (58.3)	32 (66.7)	13 (76.5)	106 (44.2)
Graft loss	8 (6.5)	6 (5.2)	6 (6.3)	5 (7.7)	3 (5)	2 (4.2)	2 (11.8)	14 (5.8)
Death	6 (4.8)	6 (5.2)	3 (3.2)	2 (3.1)	2 (3.3)	2 (4.2)	0 (0)	12 (5)

ACR, acute rejection; BKV, BK virus; BMI, body mass index; CNI, calcineurin inhibitor; CsA, cyclosporine A; DGF, delayed graft function; ECD, extended-criteria donors; IL-2R, interleukin-2 receptor; MMF, mycophenolate mofetil; MPA AUC, mycophenolic acid area under the curve; PRA, panel-reactive antibody; PyVAN, polyomavirus-associated nephropathy; T0, trough level.

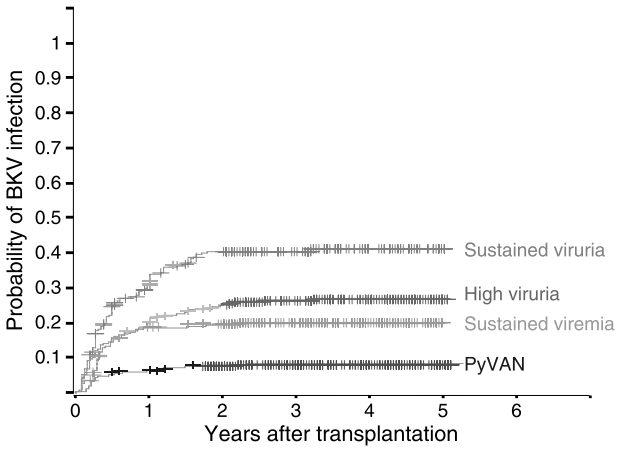

FIGURE 1. Cumulative incidence of sustained viruria, high-level viruria, sustained viremia, and PyVAN in kidney transplant or pancreas-kidney recipients according to Kaplan–Meier analysis.

(AHR, 3.01; P=0.05). When each calcineurin inhibitor was analyzed separately, MPA AUC more than 50 hr mg/L was associated with BK sustained viremia both among patients receiving CsA (AHR, 4.4; P=0.034) and in those treated by tacrolimus (AHR, 2.6; P=0.02).

DISCUSSION

In our study, we found that nearly half of kidney transplant recipients had BKV, one quarter had BKV, and 7% had biopsy-proven PyVAN. These data are consistent with previous findings (*13*). We identified factors associated with a higher risk of BKV infection. Prolonged CIT was associated with viremia and PyVAN, and DGF was a risk factor for sustained viremia. Compared with other studies, the mean CIT was higher in our series because we had few living donors, which may also explain our relatively higher rate of DGF episodes. The influence of DGF and CIT on BKV infection is controversial in the literature, in which some authors found a correlation (*23, 27*) and others did not (*11, 16, 17, 28, 29*).

Interestingly, we found a strong association between CMV mismatch and BKV viremia and PyVAN. This has never been described before and could be due to a surrogate marker of donor/recipient BKV serostatus, as has been shown for CMV and EBV serostatus (*30*). Indeed, we could speculate that CMV-negative recipients could be more frequently negative for BKV at the time of transplantation. Ali et al. (*31*) showed that recipient-negative BKV serostatus is an important risk factor for developing BKV infection after transplantation.

We found a relationship between retransplantation and sustained viremia likely because high-risk patients received a more potent immunosuppressive treatment. In our study, PRA more than 0% was correlated with an increased risk of viremia and PyVAN. This link was not found in a previous study (*16*); however, the authors compared PRA 10% and less than 10%, whereas PRA more than 0% were compared with no PRA in our study. In our series, induction with ATG was a risk factor for sustained viremia but not PyVAN, as described by Dharnidharka et al. (*23*). Hirsch et al. (*13*) did not find a link between induction therapy and BKV infection; however, in that study, ATG and basiliximab were compared together versus no induction. In our series, acute rejection in the first 3 months was an important risk factor for sustained viremia and PyVAN (*16, 17, 29*), most likely because immunosuppressive treatment is largely intensified in recipients who develop acute rejection.

One of our objectives was to study the link between exposure to immunosuppressive drugs and BKV reactivation. In our series, no association was found between steroid treatment and BK infection, in contrast to Dadhania et al. (*32*) who found an odds ratio of 8.27 for BKV replication in patients with a steroid maintenance regimen; however, these authors did not include tacrolimus and MMF therapy in their multivariate logistic regression model. We observed a link between tacrolimus and viremia and PyVAN, especially when trough levels were greater than 10 ng/mL at 3 months after transplantation. Koukoulaki et al. (*33*) reported a higher rate of viremia in recipients treated with tacrolimus compared with those treated with CsA. Cosio (*34*) reported a higher incidence of PyVAN in patients with high tacrolimus trough levels (>8 ng/mL during the first year). Similarly, patients with PyVAN had significantly higher tacrolimus trough levels in the series reported recently by Manitpisitkul et al. (*26*).

In our series, we found a significant association between MMF exposure evaluated by AUC at M3 and BKV replication. A M3 MPA $AUC_{0-12\ hr}$ of more than 50 hr mg/L was linked with sustained viremia (AHR, 3.6; P<0.001) and PyVAN (AHR, 3.01; P=0.05). As expected in light of the pharmacokinetic interaction between tacrolimus and MPA, we found that patients treated with the association tacrolimus+MMF had a mean M3 MPA AUC significantly higher than those treated with CsA and MMF. However, we showed that an elevated MPA AUC was similarly associated with BKV infection also in patients treated with CsA+MMF. This is the first time that a study has shown this relationship, and this highlights the importance of monitoring MMF routinely. Previous studies of therapeutic monitoring of MMF have shown a good correlation between the total MPA AUC and risk of acute rejection but did not investigate viral replication (*35, 36*). In line with previous studies (*5, 26*), we found no link between MMF doses and BKV replication. This highlights the importance of monitoring MPA AUC not only to avoid rejection but also to limit viral replication, particularly BK infection.

In our series, patients treated with the combination of tacrolimus and MMF had the highest incidence of viremia and PyVAN, especially when the M3 MPA $AUC_{0-12\ hr}$ was greater than 50 hr mg/L and the tacrolimus trough level at M3 was greater than 10 ng/mL (56% sustained viremia and 22% PyVAN at 2 years). In this high-risk group, we propose to screen BKV very frequently and perform MPA $AUC_{0-12\ hr}$ regularly to adjust MMF doses.

It is widely accepted that immunosuppression should be reduced after biopsy-proven diagnosis of PyVAN and even earlier when viremia is high (>10^4 copies/mL) to prevent irreversible lesions. Because our study showed that

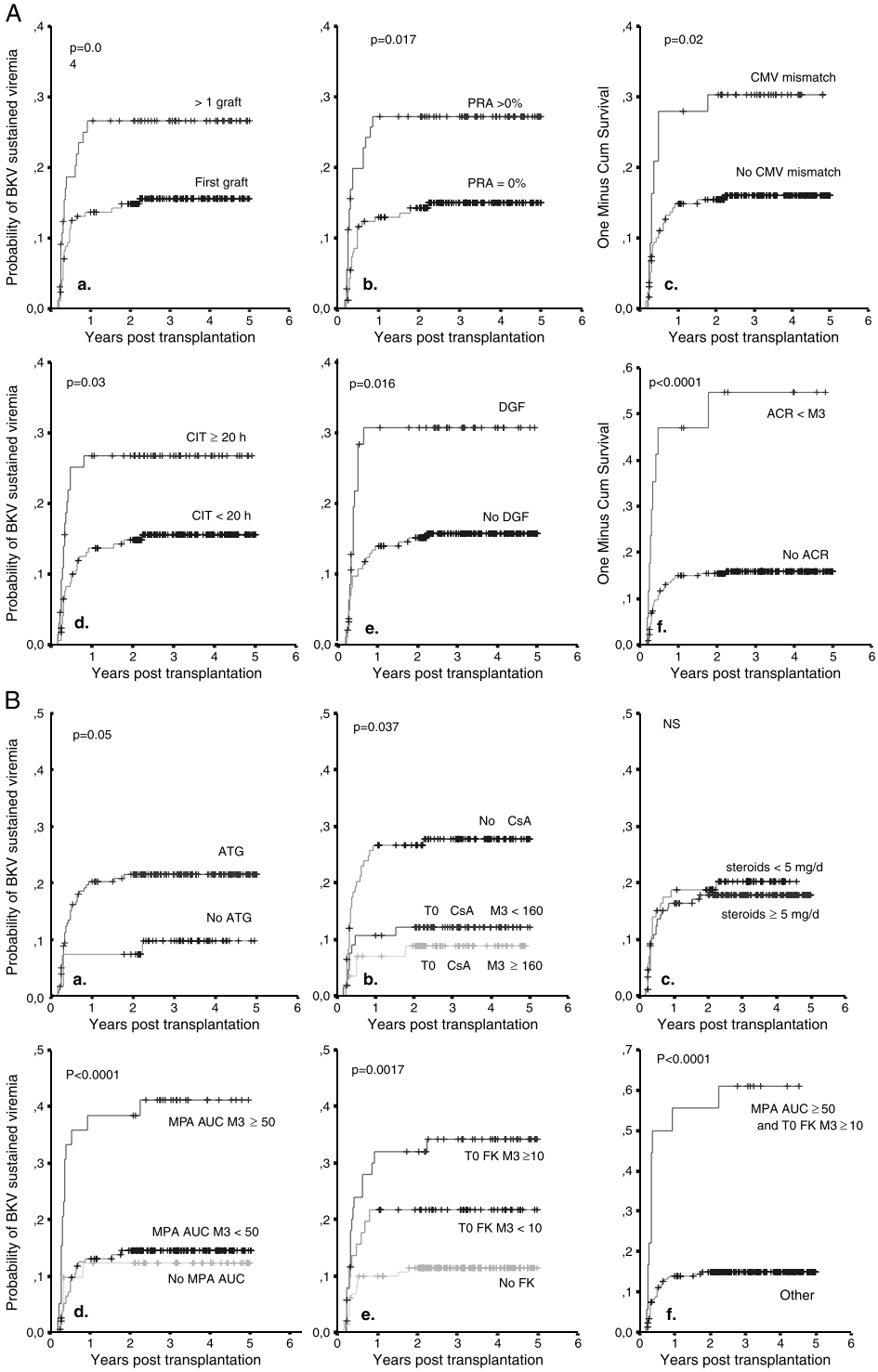

FIGURE 2. A, cumulative incidence of sustained viremia according to the following factors: (a) rank of graft, (b) PRA level, (c) donor/recipient CMV mismatch, (d) CIT, (e) DGF, and (f) occurrence of ACR in the first 3 months (Kaplan–Meier analysis). Groups were compared by the log-rank test. B, cumulative incidence of sustained viremia according to the following factors: (a) induction with ATG, (b) trough level of CsA at M3 (T0 ≥160 ng/mL [n=59], T0 <160 ng/mL [n=66], "No CsA"=missing T0 [n=3], and patients without CsA [n=108]), (c) maintenance therapy with steroids at M3 (≥5 mg/day [n=148] and <5 mg/day [n=88]), (d) MPA $AUC_{0-12\ hr}$ at M3 (MPA AUC ≥50 hr mg/L [n=39], MPA AUC <50 hr mg/L [n=156], "No MPA AUC"=missing AUC [n=23], and patients without MMF [n=18]), (e) trough level of tacrolimus at M3 (T0 ≥10 ng/mL [n=51], T0 <10 ng/mL [n=52], and "No FK"=patients without tacrolimus [n=133]), and (f) MPA $AUC_{0-12\ hr}$ and tacrolimus trough level at M3 (MPA AUC ≥50 hr mg/L and T0 FK≥10 ng/mL [n=18] and all others [n=218]; Kaplan–Meier analysis). Groups were compared by log-rank test.

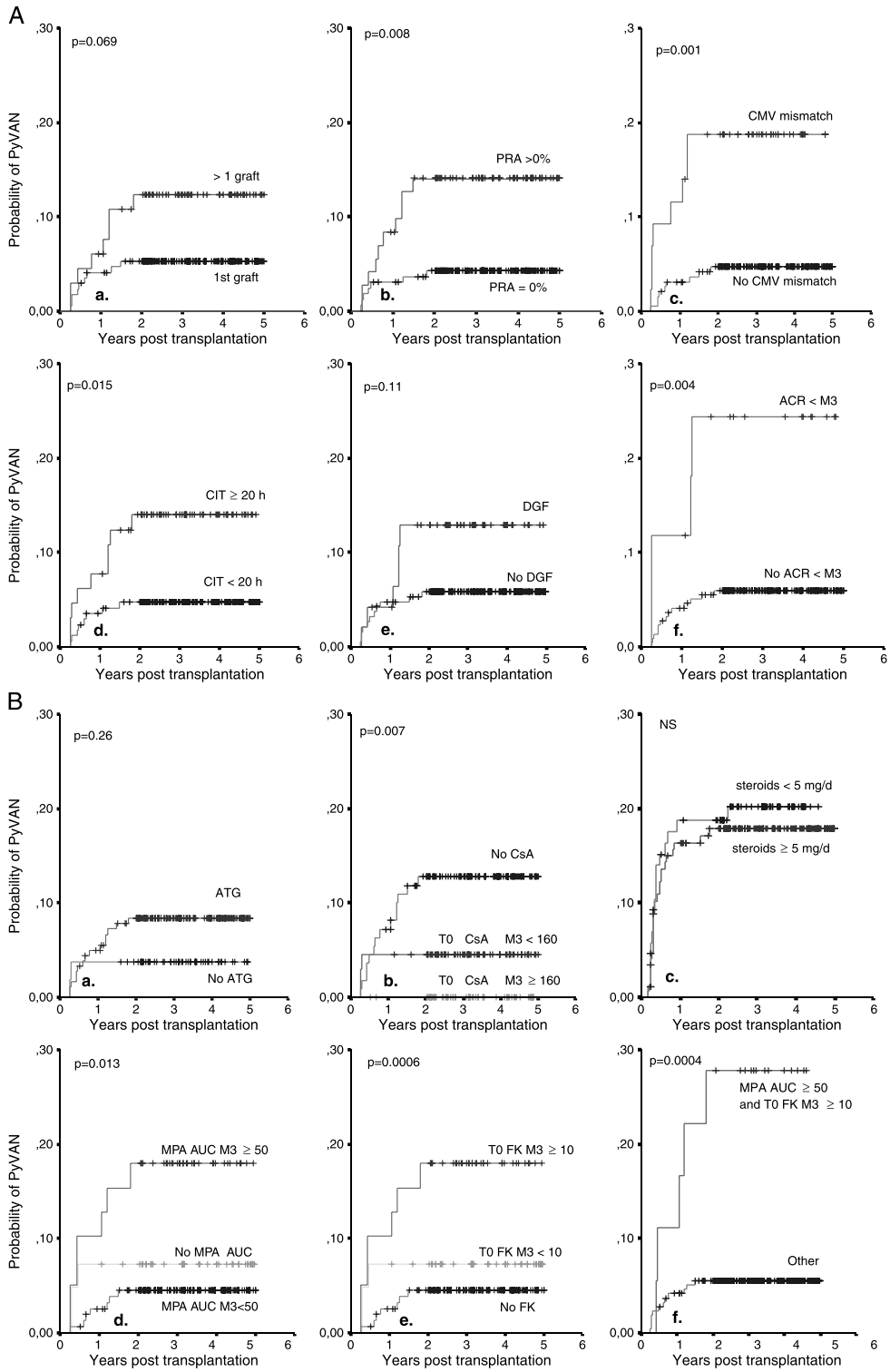

FIGURE 3. A, cumulative incidence of PyVAN according to the following factors: (a) rank of graft, (b) PRA level, (c) donor/recipient CMV mismatch, (d) CIT, (e) DGF, and (f) occurrence of ACR in the first 3 months (Kaplan–Meier analysis). Groups were compared by log-rank test. B, cumulative incidence of PyVAN according to the following factors: (a) induction with ATG, (b) trough level of CsA at M3 (T0 ≥160 ng/mL [n=59], T0 <160 ng/mL [n=66], "No CsA"=missing T0 [n=3], and patients without CsA [n=108]), (c) maintenance therapy with steroids at M3 (≥5 mg/day [n=148] and <5 mg/day [n=88]), (d) MPA $AUC_{0-12\ hr}$ at M3 (MPA AUC ≥50 hr mg/L [n=39], MPA AUC <50 hr mg/L [n=156], "No MPA AUC"=missing AUC [n=23], and patients without MMF [n=18]), (e) trough level of tacrolimus at M3 (T0 ≥10 ng/mL [n=51], T0 <10 ng/mL [n=52], and "No FK"=patients without tacrolimus [n=133]), and (f) MPA $AUC_{0-12\ hr}$ and tacrolimus trough level at M3 (MPA AUC ≥50 hr mg/L and T0 FK ≥10 ng/mL [n=18] and all others [n=218]; Kaplan–Meier analysis). Groups were compared by log-rank test.

TABLE 2. Multivariable analysis: risk factors for BKV sustained viremia and PyVAN

	BKV sustained viremia			PyVAN		
	P	AHR	95% CI	*P*	AHR	95% CI
Rank of graft >1	0.69	0.82	0.3–2.2	0.16	0.31	0.6–1.6
PRA >0%	0.16	1.86	0.9–4.4	0.15	2.84	0.7–11.7
DGF	0.25	1.51	0.8–3.1	0.91	1.07	0.3– 3.8
CIT >20 hr	0.33	1.38	0.7–2.6	0.18	2.11	0.7–6.3
ATG	0.16	2.01	0.8–5.4	0.83	1.19	0.2–6
CMV mismatch	0.05	2.03	1–4.1	0.01	4.17	1.4–12.5
ACR before M3	<0.001	5.4	2.2–12.8	0.43	1.75	0.4–7.1
T0 FK M3 >10 ng/mL	0.32	1.48	0.7–3.2	0.27	2.17	0.97–9.3
MPA AUC M3 >50 hr mg/L	0.001	3.6	1.8–7.1	0.05	3.01	1–9.3

ACR, acute rejection; AHR, adjusted hazard ratio; ATG, antithymocyte globulins; BKV, BK virus; CI, confidence interval; CIT, cold ischemia time; CMV, cytomegalovirus; CMV mismatch, D+/R-, DGF, delayed graft function; MPA AUC, mycophenolic acid area under the curve at month 3; PRA, panel-reactive antibody; PyVAN, polyomavirus-associated nephropathy; T0 FK, trough level of tacrolimus at M3.

PyVAN was significantly correlated with MPA $AUC_{0-12\ hr}$ more than 50 hr mg/L in the first 3 months, we recommend a target MPA $AUC_{0-12\ hr}$ of 40 hr mg/L rather than 50 hr mg/L or more. In cases of sustained BKV, we suggest a lower target of 20 hr mg/L.

Because our study was retrospective, some data are lacking because after 3 months, the patients were followed either in our center or other centers or patients missed their appointments. Among the 222 patients treated with MMF at M3, the AUC was lacking for 25 (11%), including 14 who presented with BKV viruria and 5 with BKV viremia.

In summary, our results demonstrated a significant correlation between BKV infection, high MMF exposure, and high tacrolimus trough levels at M3 after transplantation. This observation suggests that monitoring MPA AUC should be performed routinely to adjust MMF doses adequately to balance rejection and viral replication risks. These findings must be confirmed in prospective studies.

MATERIALS AND METHODS

We retrospectively enrolled all adult kidney and kidney-pancreas recipients with a functioning graft at 3 months transplanted at Strasbourg University Hospital between January 2006 and December 2008.

After transplantation, immunosuppressive treatment consisted of induction therapy with either basiliximab (Simulect; Novartis, Basel, Switzerland) or ATG (Thymoglobulins; Genzyme, Cambridge, MA), calcineurin inhibitors (CsA or tacrolimus), MMF, and steroids. Steroids were initially given at a dose of 1 mg/kg per day and then progressively tapered off during the first 4 months after transplantation. In immunologically high-risk patients or patients with a history of acute rejection, steroids were continued at a dose of 0.1 mg/kg per day. Target trough levels of tacrolimus were 10 to 12 ng/mL in the first 3 months, 8 to 10 ng/mL from 4 to 6 months, and 6 to 8 ng/mL thereafter. Target trough levels of CsA were 150 to 200 ng/mL in the first 6 months, 125 to 150 ng/mL from 6 to 12 months, and 75 to 125 ng/mL thereafter. The target for M3 MPA $AUC_{0-12\ hr}$ was 30 to 60 hr mg/L. All patients were treated with ofloxacin until the urinary catheter was removed (at day 5). In cases of acute cellular rejection, steroid pulses were administrated for 3 days, followed by oral steroids at a dose of 1 mg/kg per day, in addition to a switch to tacrolimus in patients treated with CsA.

Virologic Studies

DNA from 200 μL urine or whole blood samples was purified in a robotic workstation (QIAxtractor; Qiagen, Valencia, CA). The purified DNA was amplified using a quantitative real-time PCR assay as described previously (*6*). The quantitative range of this assay is 500 to 50,000,000 copies/mL. One negative and one positive controls were systematically included in each series (*37*). BKV was monitored by urine PCR every 2 months during the first 2 years and every 6 months thereafter. When viruria exceeded 10^6 copies/mL or in cases of graft dysfunction, blood samples were tested by PCR. A kidney biopsy was performed when viremia rose above 10^4 copies/mL or in cases of graft dysfunction. Sustained viruria was defined by at least two PCR 500 copies/mL or more. High-level viruria was defined by PCR more than 10^6 copies/mL and sustained viremia by at least two PCR 1000 copies/mL or more.

Cytologic and Histologic Studies

Definitive PyVAN was diagnosed by histology, and biopsy was graded according to the severity of viral cytopathic changes, inflammatory infiltrates, tubular atrophy, and fibrosis (*38*). Diagnosis was confirmed using immunohistochemistry with cross-reacting antibodies against the large T antigen of the related simian polyomavirus (SV40 antibody; Calbiochem, San Diego, CA).

Statistical Analysis

Kaplan–Meier analyses were used to construct survival plots of BKV replication for each group (viruria, sustained viruria, high viruria, viremia, sustained viremia, and PyVAN). The date of the event onset was assumed to be the first date of documented viruria, viremia, or PyVAN, with patients censored at death, loss to follow-up, dialysis, or at the end of the study (January 1, 2011). Associations between risk factors and severe BKV infection (sustained viremia and PyVAN) were only examined in patients with BK infection occurring after 3 months after transplantation (n=236) by unadjusted analysis for recipient age and gender, body mass index, primary kidney disease, PRA, rank of transplantation, donor type, ECD or not, CIT, DGF, number of HLA mismatches, donor/recipient CMV serostatus at the time of transplantation, CMV or EBV infection in the first 3 months, type of induction treatment, acute rejection before M3, treatment with oral steroids at M3, MPA AUC at M3, T0 tacrolimus at M3, and MMF+ tacrolimus treatment at M3. Comparisons were made using log-rank tests. Variables with $P<0.10$ in univariate analyses for relationship with sustained viremia or PyVAN occurrence were used as covariates in a multivariate Cox regression model (first or subsequent transplantation, PRA, induction therapy, CIT, DGF, donor/recipient CMV serostatus, acute rejection before M3, MPA AUC at M3, and T0 tacrolimus at M3). The results of Cox regression are presented as AHR with 95% confidence intervals. ROC curves were plotted for M3 MPA AUC and PyVAN occurrence, censored for PyVAN occurring before 3 months and after 24 months after transplantation. Statistical analyses were performed using SPSS 11.5 (SPSS, Chicago, IL). Two-tailed *P* values<0.05 were considered statistically significant.

ACKNOWLEDGMENTS

The authors thank Dr. Kemmel, Dr. Oubaasine, and Prof. Ubeaud Sequier for performing the MPA area under curve analyses, Dr. Samira Fafi Kremer and Prof. Stoll Keller for performing the BKV PCR tests in the urine and blood samples, and Prof. Hirsch for his helpful advice and supportive suggestions.

REFERENCES

1. Purighalla R, Shapiro R, McCauley J, et al. BK virus infection in a kidney allograft diagnosed by needle biopsy. *Am J Kidney Dis* 1995; 26: 671.
2. Binet I. Polyomavirus disease under new immunosuppressive drugs: a cause of renal graft dysfunction and graft loss. *Transplantation* 1999; 67: 918.
3. Howell DN, Smith SR, Butterly DW, et al. Diagnosis and management of BK polyomavirus interstitial nephritis in renal transplant recipients. *Transplantation* 1999; 68: 1279.
4. Randhawa PS, Finkelstein S, Scantlebury V, et al. Human polyoma virus-associated interstitial nephritis in the allograft kidney. *Transplantation* 1999; 67: 103.
5. Brennan DC, Agha I, Bohl DL, et al. Incidence of BK with tacrolimus versus cyclosporine and impact of preemptive immunosuppression reduction. *Am J Transplant* 2005; 5: 582.
6. Bressollette-Bodin C, Coste-Burel M, Hourmant M, et al. A prospective longitudinal study of BK virus infection in 104 renal transplant recipients. *Am J Transplant* 2005; 5: 1926.
7. Ramos E, Drachenberg CB, Wali R, et al. The decade of polyomavirus BK-associated nephropathy: state of affairs. *Transplantation* 2009; 87: 621.
8. Hirsch HH, Brennan DC, Drachenberg CB, et al. Polyomavirus-associated nephropathy in renal transplantation: interdisciplinary analyses and recommendations. *Transplantation* 2005; 79: 1277.
9. Drachenberg CB, Hirsch HH, Papadimitriou JC, et al. Polyomavirus BK versus JC replication and nephropathy in renal transplant recipients: a prospective evaluation. *Transplantation* 2007; 84: 323.
10. Randhawa P, Ho A, Shapiro R, et al. Correlates of quantitative measurement of BK polyomavirus (BKV) DNA with clinical course of BKV infection in renal transplant patients. *J Clin Microbiol* 2004; 42: 1176.
11. Ramos E. Clinical course of polyoma virus nephropathy in 67 renal transplant patients. *J Am Soc Nephrol* 2002; 13: 2145.
12. Bohl DL, Storch GA, Ryschkewitsch C, et al. Donor origin of BK virus in renal transplantation and role of HLA C7 in susceptibility to sustained BK viremia. *Am J Transplant* 2005; 5: 2213.
13. Hirsch HH, Knowles W, Dickenmann M, et al. Prospective study of polyomavirus type BK replication and nephropathy in renal-transplant recipients. *N Engl J Med* 2002; 347: 488.
14. Shah KV. Human polyomavirus BKV and renal disease. *Nephrol Dial Transplant* 2000; 15: 754.
15. Nickeleit V, Hirsch HH, Zeiler M, et al. BK-virus nephropathy in renal transplants—tubular necrosis, MHC-class II expression and rejection in a puzzling game. *Nephrol Dial Transplant* 2000; 15: 324.
16. Adawalla Y. HLA mismatching increases the risk of BK virus nephropathy in renal transplant recipients. *Am J Transpl* 2004; 4: 1691.
17. Mengel M. Incidence of polyomavirus-nephropathy in renal allografts: influence of modern immunosuppressive drugs. *Nephrol Dial Transplant* 2003; 18: 1190.
18. White LH, Casian A, Hilton R, et al. BK virus nephropathy in renal transplant patients in London. *Transplantation* 2008; 85: 1008.
19. Randhawa P, Brennan DC. BK virus infection in transplant recipients: an overview and update. *Am J Transplant* 2006; 6: 2000.
20. Hurault de Ligny B, Etienne I, Francois A, et al. Polyomavirus-induced acute tubulo-interstitial nephritis in renal allograft recipients. *Transplant Proc* 2000; 32: 2760.
21. Hirsch H. Prospective monitoring of BK virus load after discontinuing sirolimus treatment in a renal transplant patient with BK virus nephropathy. *J Infect Dis* 2001; 184: 1494.
22. Lipshutz GS, Flechner SM, Govani MV, et al. BK nephropathy in kidney transplant recipients treated with a calcineurin inhibitor-free immunosuppression regimen. *Am J Transplant* 2004; 4: 2132.
23. Dharnidharka VR, Cherikh WS, Abbott KC. An OPTN analysis of national registry data on treatment of BK virus allograft nephropathy in the United States. *Transplantation* 2009; 87: 1019.
24. Smith JM, Dharnidharka VR, Talley L, et al. BK virus nephropathy in pediatric renal transplant recipients: an analysis of the North American Pediatric Renal Trials and Collaborative Studies (NAPRTCS) registry. *Clin J Am Soc Nephrol* 2007; 2: 1037.
25. Trofe J. Polyomavirus in kidney and kidney-pancreas transplantation: a defined protocol for immunosuppression reduction and histologic monitoring. *Transpl Proc* 2002; 34: 1788.
26. Manitpisitkul W, Drachenberg C, Ramos E, et al. Maintenance immunosuppressive agents as risk factors for BK virus nephropathy: a case-control study. *Transplantation* 2009; 88: 83.
27. Vasudev B, Hariharan S, Hussain SA, et al. BK virus nephritis: risk factors, timing, and outcome in renal transplant recipients. *Kidney Int* 2005; 68: 1834.
28. Ginevri F, Azzi A, Hirsch HH, et al. Prospective monitoring of polyomavirus BK replication and impact of pre-emptive intervention in pediatric kidney recipients. *Am J Transplant* 2007; 7: 2727.
29. Schold JD, Rehman S, Kayle LK, et al. Treatment for BK virus: incidence, risk factors and outcomes for kidney transplant recipients in the United States. *Transpl Int* 2009; 22: 626.
30. Cherikh WS, Kauffman HM, McBride MA, et al. Association of the type of induction immunosuppression with posttransplant lymphoproliferative disorder, graft survival, and patient survival after primary kidney transplantation. *Transplantation* 2003; 76: 1289.
31. Ali AM, Gibson IW, Birk P, et al. Pretransplant serologic testing to identify the risk of polyoma BK viremia in pediatric kidney transplant recipients. *Pediatr Transplant* 2011; 15: 827.
32. Dadhania D, Snopkowski C, Ding R, et al. Epidemiology of BK virus in renal allograft recipients: independent risk factors for BK virus replication. *Transplantation* 2008; 86: 521.
33. Koukoulaki M, Grispou E, Pistolas D, et al. Prospective monitoring of BK virus replication in renal transplant recipients. *Transpl Infect Dis* 2009; 11: 1.
34. Cosio FG. Comparison of low versus high tacrolimus levels in kidney transplantation: assessment of efficacy by protocol biopsies. *Transplantation* 2007; 83: 411.
35. Knight SR. Does the evidence support the use of mycophenolate mofetil therapeutic drug monitoring in clinical practice? A systematic review. *Transplantation* 2008; 85: 1675.
36. LeMeur Y. Individualized mycophenolate mofetil dosing based on drug exposure significantly improves patient outcomes after renal transplantation. *Am J Transpl* 2007; 7: 2496.
37. Fafi-Kremer S. Evaluation of the Epstein-Barr virus R-Gene Quantification Kit in whole blood with different extraction methods and PCR platforms. *J Mol Diagn* 2008; 10: 78.
38. Drachenberg CB, Hirsch HH, Ramos E, et al. Polyomavirus disease in renal transplantation: review of pathological findings and diagnostic methods. *Hum Pathol* 2005; 36: 1245.