Moscarelli et al., J Nephrol Therapeutic 2012, S4
DOI: 10.4172/2161-0959.S4-005

Journal of Nephrology & Therapeutics

Research Article Open Access

BKV-DNA Replication in Renal Transplant Recipients: Early Discontinuation of Mycophenolate Mofetil or an Early Combination Therapy with Fluoroquinolones and Activated Vitamin D. What Is the Best Strategy for Management?

Luciano Moscarelli*, Anduela Mjeshtri, Filomena Annunziata, Aris Tsalouchos, and Elisabetta Bertoni

Renal Unit Careggi University Hospital, Florence, Italy

Abstract

Background: Early detection of polyomavirus BK-viremia and an early reduction of the maintenance immunosuppressive therapy are recommended for preventing polyomavirus-associated-nephropathy, but despite these measures, a progressive graft dysfunction and a graft loss occur in many cases.

Objectives: We diagnosed BKV-viremia in 92 recipients grafted between October 2005 and January 2010. 53 affected recipients were treated with only a discontinuation of mycophenolate mofetil (Group 1). 39 affected recipients were treated with a combination therapy based on fluoroquinolones and activated vitamin D without a reduction of the maintenance immunosuppression (Group 2). All recipients were studied for a minimum follow-up period of 18 months after diagnosis of BKV-viremia.

Results: The mean blood viral load during follow-up decreased in both groups but the graft function was stabilized only in Group 2. In fact in Group 2 the mean creatinine clearance was 68.5 +/- 18.1 ml/min after follow-up versus 69.1 +/- 25 ml/min before diagnosis of BKV-viremia (p = ns) and the mean serum creatinine was 1.31 +/- 1.1 mg/dl versus 1.27 +/- 0.9 mg/dl before diagnosis of BKV-viremia (p = ns). Group 2 affected recipients had 13% major graft functional decline rate (5 affected recipients had doubling of serum creatinine after the follow-up) while Group 1 affected recipients had 36% major graft functional decline rate (7 affected recipients had doubling of serum creatinine and 12 affected recipients had graft loss after the follow-up). The rate of polyomavirus-associated-nephropathy was similar between the groups: 9.4% in Group 1 versus 10% in Group 2 (p = ns). 7 affected recipients of Group 1 developed a new acute rejection episode during the minimum follow-up. None new acute rejection episode occurred in affected recipients of Group 2 during the minimum follow-up. Risk factors were also evaluated.

Conclusion: In this select group of renal transplant recipients with BKV-replication the use of a combination therapy based on fluoroquinolones and activated vitamin D resulted in prolonged graft survival and stabilized graft function than an early discontinuation of mycophenolate mofetil.

Keywords: BK polyomavirus; Calcitriol; Interstitial nephritis; Fluoroquinolones; Renal transplantation

Introduction

Polyomavirus BK (BKV) replication has been identified as an important cause of interstitial nephritis and of graft loss in renal transplant recipients (RTRs) [1,2]. The estimated prevalence of polyomavirus associated nephropathy (PVAN) is between 1-10 % of all RTRs [2]. The introduction in clinical practice of newer immune suppressive agents, such as tacrolimus (Fk) and mycophenolate mofetil (MMF) has resulted in a substantial reduction of acute rejection (AR) rates but it has coincided with re-emergence of PVAN [1]. BK-viruria is the first sign of active virus replication and the progression to BK-viremia is the prerequisite for the development of PVAN. The gold standard for diagnosis of PVAN continues to be biopsy [2] supported by use of immunohistochemistry for large T-antigen. Because PVAN is presents histologically as a focal disease, false-negative biopsy results have been estimated to occur in 10-30% of cases. It has been demonstrated that BKV replication in the allograft has been correlated with the detection of BKV load in blood [3,4]. In asymptomatic patients or with increased serum creatinine (sC), with persisting high-level BKV viremia, with > 10^4 copies/ml plasma for a time > three weeks and with a negative biopsy result, the diagnosis of presumptive-PVAN was been proposed [2]. So the positive polymerase chain reaction (PCR) BKV in plasma is a sensitive and specific methods for early identification of the reactivation viral infection and then of viral nephropathy. The early reduction of the maintenance immunosuppressive therapy in RTR with BKV-viremia, is reported by some authors with a safe and effective intervention strategy. There are some strategies: a reduction/discontinuation of the anti-proliferative drugs (MMF or azathioprine) or a reduction of the calcineurin-inhibitors (CyA or Fk), or a reduction of the total maintenance immunosuppression by 25-50% or the switch of immunosuppressant (from Fk to CyA) [1,5-8]. However, this strategy may increase the risk of AR or predispose to chronic allograft nephropathy [2]. In patients with a progressive graft dysfunction who have not responded to this strategy, antiviral treatments have been proposed. Ancillary therapy with antiviral agents such as cidofovir, leflunomide, and intravenous immunoglobulin, are not clear as there is a paucity of data and there are no randomized clinical trials. Moreover it should be notes that these reports have been complicated by the fact that the administration of this antiviral agents was also

***Corresponding author:** Dr. Luciano Moscarelli, Renal Unit Careggi University Hospital, Viale Pieraccini 18, 50139 Florence Italy, E-mail: moscarellil@libero.it

Received August 12, 2011; **Accepted** March 22, 2012; **Published** March 24, 2012

Citation: Moscarelli L, Mjeshtri A, Annunziata F, Tsalouchos A, Bertoni E (2012) BKV-DNA Replication in Renal Transplant Recipients: Early Discontinuation of Mycophenolate Mofetil or an Early Combination Therapy with Fluoroquinolones and Activated Vitamin D. What Is the Best Strategy for Management? J Nephrol Therapeutic S4:005. doi:10.4172/2161-0959.S4-005

done simultaneously with immunosuppression reductions, making it difficult to comment on the true effectiveness of the pharmacological intervention. Fluoroquinolones display an anti-BK properties through inhibition of DNA topoisomerase and polyomavirus associated large T-antigen helicase [9]. Fluoroquinolones to obstruct simian virus (SV40) polyomavirus replication in vitro [9]. Chandraker et al. [10] showed the positive effect of a short course of gatifloxacin on RTRs BKV-urine excreting. Moreover, exposure to ciprofloxacin seems to decrease the BKV load in another study reported in bone marrow transplant recipients [11]. Recently Gabardi et al. [12] have showed a preventing effect of fluoroquinolones on BKV-viremia after renal transplant (RT). The vitamin D system had multiple physiological and pharmacological effects mediated by action of the vitamin D receptors (VDRs). Recently, VDR activators (VDRA) have been shown to obstruct the cell replication and have an immunomodulatory properties. An important observation was reported which suggested that toll-like receptors (TLRs) activation of human macrophages upregulated expression of vitamin D receptor and vitamin D-1-hydroxylase genes, leading to induction of the antimicrobial peptide [13]. This suggests an association of TLRs and vitamin D-mediated innate-immunity [13] which represent a first line of defence against infection. In the literature, there are no specific works on the interference of vitamin D activated against BKV-infection but are reported its interferences against other microorganisms (mycobacterium tuberculosis, influenza virus, hepatitis C virus) [14,15,16]. On the basis of this researches we have evaluated after a minimum follow-up period of 18 months after diagnosis of BKV-viremia, the safety and efficacy of a new management protocol for to reduce BKV-replication based on the use of fluoroquinolones and vitamin D combination compared with an our precedent protocol based on an early MMF discontinuation. The secondary purpose was to evaluate the various risk factors for BKV-infection.

Materials and Methods

This is a single-center study conducted on 347 RTRs who were admitted to our institution from October 2005 to January 2010. Renal transplants performed after January 2010 were not included, for to ensure that all patients had a minimum follow-up of 18 months after diagnosis of BKV-viremia. All patients were adult (age > 18 years) and were treated in the Renal Unit with follow-up control visits planned according to American Society of Nephrology guidelines [17]. All clinical and laboratory data concerning RTRs admitted to our Renal Unit since 2005 were registered as routine practice at our institution. This study was conducted according to Institutional guidelines, and patients signed an informed consent before entering the study. We used a strategy to detect BKV- DNA in urine and in blood by using PCR tests weekly during the first 4 months post RT, then monthly. Patients were considered to have BKV-viremia/viruria if their viral load in blood/urine was > 850 copies/ml. Definitive PVAN was diagnosed with biopsy specimens (two cores obtained with a 16 gauge needle) and evaluated by light microscopy and immunohistochemistry method (SV40 large T-antigen). All graft biopsies contained areas both cortex and medulla zone. Findings were graded according to the Drachenberg et al. [18] classification. In asymptomatic patients or with increased of sC with persisting high-level BKV-viremia, with > 10^4 copies/ml plasma for a time > three weeks, and with a negative graft biopsy the diagnosis of presumptive-PVAN was made. The mean age at RT was 55 (range 49-69) years in Group1 and 50 (range 27-69) years in Group 2 (p = ns). At diagnosis of BKV-viremia 30 (57%) of 53 affected Group 1 and 23 (54%) of 39 affected Group 2 had still cytomegalovirus (CMV) prophylaxis/treatment. Since May 2006 we used universal 3-months prophylaxis with acyclovir to prevent CMV disease. Then prophylaxis with valganciclovir for 3 months after RT was used only in subset of high-risk independent of viral replication (CMV donor-positive/recipient-negative or patients treated with ATG). An antimicrobial prophylaxis with cephalosporin was initiated within the first 12 h after surgery. All patients received co-trimoxazole one single daily dose for six months for Pneumocystis Carinii pneumonia prophylaxis and oral washing with nystatin for three months for to prevent oral or gastrointestinal fungal infections. According to our local immunosuppression policy, all patients received induction-therapy with basiliximab before surgery. It was administered as follows: 1 dose of 20 mg intravenously on day 0 and 4 after RT. During the surgical procedure 500 mg of methylprednisolone was administered intravenously. 298 RTRs were treated with a cyclosporine A (CyA), steroid and MMF regimen. CyA dose was adjusted to maintain trough blood levels between 150-300 ng/ml until 6 months after RT and then between 70-150 ng/ml. Steroid (methylprednisolone) dose was 16 mg/day until the third month after RT an then progressively decreased to 8 mg/day at 6 months after RT. MMF dose was 2 g/day. 49 RTRs were treated with Fk, MMF and steroid (this recipients had higher immunological risk as with > 1 HLA A or B antigen mismatch, previous transplant, panel reactive antibody > 20% or they had experiencing acute rejection with apparently adequate CyA blood concentration and they afterwards were switched from CyA to Fk). Fk dose was adjusted to maintain trough blood levels between 7-10 ng/ml until 3 months after RT and then between 4-7 ng/ml. MMF dose was 1 g/day. Steroid (methylprednisolone) dose was 16 mg/day until 1 month after RT and then progressively decreased to 4 mg/day. Till April 2008 in every patient with BKV-viremia > 850 copies/ml plasma for a time > three weeks MMF was immediately discontinued (53 recipients, Group 1), since April 2008 every patient with BKV-viremia > 850 copies/ml plasma for a time > three weeks was treated with a combination oral therapy based on levoxacin 500 mg/day for 30 days and calcitriol 1μg/day for three months (39 recipients, Group 2), while the maintenance immunosuppression was not changed. Potential side effects were systematically assessed. This included measurements of haemoglobin levels, platelet counts, liver enzymes levels, bilirubin, plasma calcium, serum phosphate, parathyroid hormone. In 2 cases with plasma calcium levels >11 mg/dl, calcitriol daily dosage was decreased by 25-50%. AR episodes were confirmed using percutaneous transplant biopsy and were scored according to the Banff classification [19]. The therapy for Banff Grade I rejection, consisted of three bolus (500 mg each) of methylprednisolone for 3 alternate days. Banff Grade II and III, as well as steroid resistant rejection, were treated with antithymocyte globulin (ATG) (1.5 mg/kg/day for seven days).

Statistical Analysis

Statistical analysis was performed using the NCSS system (NCSS, Cary, NC). Baseline characteristics (donor sex, recipient sex, donor age, recipient age, primary renal diagnosis, duration and type of pre-transplant dialysis, donor source, history of CMV prophylaxis/treatment, history of AR, type of maintenance immunosuppression) in two groups, were compared. Data are expressed as means +/- SD or as median and range, as appropriate. For the univariate comparisons t-test of mean, Chi-squared test, were applied as appropriate. A multivariate analysis using a Cox proportional hazards model was performed for to evaluate the risk factors, using time to BKV-viremia as the dependent outcome and donor sex, recipient sex, donor age, recipient age, primary renal diagnosis, duration and type of pre-transplant dialysis, donor source, history of CMV prophylaxis/treatment, history of AR, type of maintenance immunosuppression as the independent covariates, all factors that were found to be significantly predictive in preceding univariate analyses. For to evaluate the graft outcome, a

multifactorial Cox regression model was performed after the diagnosis of BKV-viremia using time to major graft functional decline (defined by graft loss or doubling of serum creatinine after the diagnosis of BKV-viremia) as dependent variable and graft outcome of Group 2 versus Group 1 and all covariates of previous multivariate analysis as the independent covariates. The Kaplan-Meier analysis was used to estimate the probability of major graft functional decline using the date of BKV-viremia diagnosis as the starting of the curve. Differences between survival curves were tested with a log-rank test. The p values < 0.05 were considered statistically significant.

Results

7 recipients were excluded from further analysis because the transplant failed within 2 weeks after surgery, giving insufficient time to initiate BKV surveillance: 3 failed on the first day for technical reasons, 1 failed because of RT vein thrombosis 3 days after, and 3 failed 6 days after because of severe AR. The statistical comparison of all demographic and clinical characteristics showed no significant differences between the two groups (Table 1). The mean recipient age at RT was 55 (range 49-69) years in Group 1 and it was 50 (range 27-69) years in Group 2 (p = 0.57). The mean donor age was 60 (range 50-72) years in Group 1 and it was 58 (range 22-73) years in Group 2 (p = 0.82). The mean duration of pre-transplant dialysis was 35 (range 10-54) months in Group 1 and it was 36 (range 3-60) months in Group 2 (p = 0.59). 40 (75%) affected recipients of Group 1 and 28 (72%) affected recipients of Group 2 were on hemodialysis (p = 0.79), 13 (25%) affected recipients of Group1 and 11 (28%) affected recipients of Group 2 were on peritoneal dialysis (p = 0.48). In Group 1 female donors were 124 (63%) and in Group 2 they were 90 (63%) (p = 0.25). In Group 1 male donors were 73 (37%) and in Group 2 they were 53 (37 %) (p = 0.48). In Group 1 affected female recipients were 25 (47.2%) and in Group 2 they were 18 (46.2%) (p = 0.37). In Group 1 affected male recipients were 28 (52.8%) and in Group 2 they were 21 (53.8%) (p = 0.36). In Group 1 affected deceased donor were 51 (96%) and in Group 2 they were 37 (95%) (p = 0.44). In Group 1 affected living donor were 2 (4%) and in Group 2 they were 2 (3%) (p = 0.38). Affected recipients with an immunosuppressive therapy based on Cya, MMF, and Cs in Group 1 were 33 (62%) and in Group 2 they were 24 (62%) (p = 0.57). Affected recipients with an immunosuppressive therapy based on Fk, MMF, and Cs in Group 1 were 20 (38%) and in Group 2 they were 15 (38%) (p = 0.84). A history of CMV prophylaxis/treatment before diagnosis of BKV-viremia was present in 30 (57%) affected recipients of Group 1 and it was present in 23 (54%) affected recipients of Group2 (p = 0.73). AR episodes before diagnosis of BKV-viremia in Group 1 were 32 (16.2%) and they were 24 (16.8%) in Group 2 (p = 0.38). Also the evaluation of some of the secondary BKV outcomes showed similar results among the groups (Table 3): the mean sC before diagnosis of BKV-viremia was 1.36 +/- 0.8 mg/dl in Group 1 and it was 1.27 +/- 0.9 mg/dl in Group 2 (p = ns). The mean creatinine clearance (CCr) before diagnosis of BKV-viremia was 71.6 +/- 30.5 ml/min in Group 1 and it was 69.1 +/- 25 ml/min in Group 2 (p = ns). The mean sC at diagnosis of BKV-viremia was 1.64 +/- 1.1 mg/dl in Group 1 and it was 1.6 +/- 1.22 mg/dl in Group 2 (p = ns). The mean CCr at diagnosis of BKV-viremia was 47.4 +/- 20.7 ml/min in Group 1 and it was 40.6 +/- 17.5 ml/min in Group 2 (p = ns). The mean blood viral

	All RT (n = 340) A U	RT till April 2008 (n = 197) Group 1	RT since April 2008 (n = 143) Group 2	p
	92 (27) 248(73)	53 (26.9)	39 (27.3)	
Donor gender Female Male	214 (58) 126 (42)	124 (63) 73 (37)	90 (63) 53 (37)	0.25 0.48
Recipient gender Female Male	43 (47) 109(44) 49 (53) 139 (56)	25 (47.2) 28 (52.8)	18 (46.2) 21 (53.8)	0.37 0.36
Recipient age at transplantation (years)	51 (23-70)	55 (49-69)	50 (27- 69)	0.57
Donor age (years)	63 (38-80)	60 (50- 72)	58 (22-73)	0.82
Renal disease Glomerulonephritis Interstitial nephritis Polycystic kidney disease Unspecified	38 (41) 107 (43) 20 (22) 54 (22) 18 (20) 50(20) 16 (17) 37 (15)	21 (40) 11 (20) 11 (20) 10 (19)	16 (41) 9 (23) 8 (20) 6 (15)	0.38 0.55 0.81 0.38
Duration of dialysis median months (range)	38 (0-60)	35 (10- 54)	36 (3- 60)	0.59
Hemodialysis	68 (74) 186 (75)	40 (75)	28 (72)	0.79
Peritoneal dialysis	24 (26) 62 (25)	13 (25)	11 (28)	0.48
Donor source Deceased donor Living donor	88 (96) 243 (98) 4 (4) 5 (2)	51 (96) 2 (4)	37 (95) 2 (3)	0.44 0.38
Treatment AR Cs ATG	46 (50) 26 (10.5) 10 (10.8) 5 (2)	26 (49) 6 (11)	20 (45) 4 (10.3)	0.38 0.36
CMV prophylaxis or treatment before diagnosis	53 (58) 27 (11)	30 (57)	23 (54)	0.73
Immunosuppressive therapy Cya, MMF, Cs (n = 295) Fk, MMF, Cs (n = 45)	57 (19) 238 (81) 35 (78) 10 (22)	33 (62) 20 (38)	24 (62) 15 (38)	0.57 0.84

The comparison of clinical characteristics between two groups did not reveal any significant differences. Fk = tacrolimus, MMF = mycophenolate mofetil, Cs = steroid, CyA = cyclosporine, ATG = antithymocyte globulins, CMV = cytomegalovirus, RT = renal transplant, A = affected, U = unaffected, AR = acute rejection.

Table 1: Baseline characteristics of the two groups, and statistical significance of the difference in clinical characteristics.

load at diagnosis in Group 1 was 14.2 +/- 7.5 x 10^4 copies/ml and it was 11.46 x 10^4 copies/ml in Group 2 (p = ns). The mean onset of BKV-viremia was 4.1 +/- 1.5 months in Group 1 and it was 4.7 +/- 1.8 months in Group 2 (p = ns) (Table 3). The results of the multivariate Cox regression analysis of possible risk factors for BKV-viremia are displayed in Table 2. Important risk factors for BKV-viremia are: female donors [Hazard ratio (Hr) = 1.73, 95% Confidence Interval (CI) = 1.05 – 2.84, p = 0.02], older RTRs (Hr = 2.16, 95% CI = 1.19 – 3.9, p = 0.01), older age donors (Hr =2.24, 95% CI = 2.1 – 4.1, p = 0.02), a history of CMV prophylaxis/treatment before the diagnosis of BKV-viremia (Hr = 2.16, 95% CI = 1.19 – 3.9, p = 0.01), deceased donor (Hr = 3.76, 95% CI = 1.02 – 13.9, p = 0.05), a precedent AR episode (Hr = 1.88, 95% CI = 1.35 – 2.61, p = 0.02), a maintenance immunosuppressive therapy based on CyA and MMF association (Hr = 2.29, 95% CI = 1.04 – 5.07, p = 0.04), or based on Fk and MMF association (Hr = 3.53, 95% CI = 1.39 – 8.94, p= 0.01). All other parameters studied were not relevant. (see Table 2 for details). The incidence of BKV-viremia during 54 months of the study was similar between the groups: in Group 1 n = 53 (26.9%) and in Group 2 n = 39 (27.3%). As for the precision of the estimates, the 95% two-side confidence interval around the proportion in Group1 is 20.8%, 33.2% and in Group 2 is 19.7%, 34.3%, showing that sample size were sufficiently large for adequately precise estimates. In addition, the samples sizes of 39 patients of Group 2 and 53 patients of Group 1 provides 92.4% power to reject the null hypothesis of equal BKV-viremia proportions.

Transplant characteristic (reference level)		Hazard ratio	95 % CI	p
Donor gender (male) Female		1.73	1.05 – 2.84	0.02
Recipient gender (male) Female		0.99	0.95 – 1.05	0.98
Recipient age at transplantation (35 – 54), years	18 – 34 55 – 64 > 64	1.19 2.16 3.53	0.34 – 4.23 1.19 – 3.9 1.39 – 8.94	0.79 0.01 0.01
Donor age (55 – 64), years	18 – 34 35 – 54 > 64	0.88 1.04 3.64	0.77 – 1.00 0.84 – 1.29 1.4 – 8.76	0.10 0.49 0.01
Renal disease (unspecified) Glomerulonephritis Interstitial nephritis Polycystic kidney disease		 1.37 1.04 0.99	 0.36 – 5.23 0.99 – 1.09 0.86 – 1.15	 0.64 0.11 0.89
Pretransplant dialysis time (3 – 10) months	11 – 54 > 54	1.38 1.16	0.55 – 3.46 0.81 – 1.67	0.50 0.55
Pretransplant dialysis type (peritoneal dialysis) Hemodialysis		0.45	0.12 – 1.79	0.26
Donor source (living donor) Deceased donor		3.76	1.02 – 13.9	0.05
Treatment AR (no treat AR) Cs ATG		 1.96 1.88	 1.33 – 2.89 1.35 – 2.61	 0.01 0.02
CMV proph/treat BD (no proph/treat) yes proph/treat		2.16	1.19 – 3.90	0.01
Immunosuppressive therapy (CyA,MMF,Cs) Fk,MMF,Cs		3.53	1.39 – 8.94	0.01

The BKV-infection incidence was significantly higher in recipients of female donor, in older recipients, in recipients of older donor, in recipients with a history of CMV prophylaxis/treatment, in recipients of deceased donor, in recipients with a history of episode of AR, in recipients with an immunosuppressive therapy based on Fk and MMF association. Fk = tacrolimus, MMF = mycophenolate mofetil, Cs = steroid, CyA = cyclosporine, ATG = antithymocyte globulins,

CMV = cytomegalovirus, AR = acute rejection, BD = before diagnosis. See text for details.

Table 2: Risk factors between two groups for BKV-viremia. Multivariate Cox regression analyses with time to BKV-viremia as the dependent variable.

	Group 1 (n = 53)	Group 2 (n = 39)	p
Mean sC +/- SD (Range 0.6- 1.2) (mg/dL) BD BL 18 months	 1.36 +/- 0.8 1.64 +/- 1.1 4.1 +/- 1.4	 1.27 +/- 0.9 1.6 +/- 1.22 1.31 +/- 1.1	 ns ns < 0.05
Mean CCr +/- SD (Range 85- 140) (ml/min) BD BL 18 months	 71.6 +/- 30.5 47.4 +/- 20.7 14.8 +/- 23	 69.1 +/- 25 40.6 +/- 17.5 68.5 +/- 18.1	 ns ns < 0.05
BKV load (x 10 ^ 4 cp/ml) BL 3 months 6 months 9 months 12 months 18 months	 14.2 +/- 7.5 11.8 +/- 5.6 8.52+/- 4.97 6.5 +/- 5.3 1.85 +/- 4.1 1.73 +/- 2.43	 11.46 +/- 7.84 3.06 +/- 2.83 1.38 +/- 0.42 1.14 +/- 0.49 1.10 +/- 0.53 1.00 +/- 0.53	 ns < 0.05 < 0.05 < 0.05 ns ns
Mean time from RT to BL (months)	4.1 +/- 1.5	4.7 +/- 1.8	ns
Mean time to clear BKV load (months)	9	3	<0.05
PVAN stage * A B	 2 2 1	 2 1 1	
AR treated FU	7	--	
Major graft functional decline (Graft loss)	19 (12)	5 (0)	
Mean time to BKV urinary shedding (months)	3.9 +/- 1.7	4 +/- 2.1	ns
Mean time to clear BKV urinary load (months)	13.6 +/- 8.3	7.3 +/- 4.5	< 0.05

At the end of follow-up the mean graft function was declined in Group 1 affected recipients and it was stable in Group 2 affected recipients. The mean time for diagnosis of BKV-viremia was similar in two groups. At diagnosis of BKV-viremia the mean blood viral load was similar in two groups. In Group 2 the larger clearance of the mean blood viral load decreased significantly since the third month after therapeutic intervention. In Group 1 the larger clearance of the mean blood viral load decreased significantly since the ninth month after therapeutic intervention. 7 affected recipients of Group 1developed a new AR episode and 19 affected recipients had a major graft functional decline (12 had graft loss and 7 had doubling of serum creatinine) after therapeutic intervention. sC = serum creatinine, CCr = creatinine clearance, SD = standard deviation, BD = before diagnosis, BL = base line, FU = follow-up, RT = renal transplant, AR = acute rejection, cp = copies, * = presumptive PVAN. Lab value in International Units; reference range in International Units.

Table 3: BKV-related outcomes in two groups of recipients.

Group 1

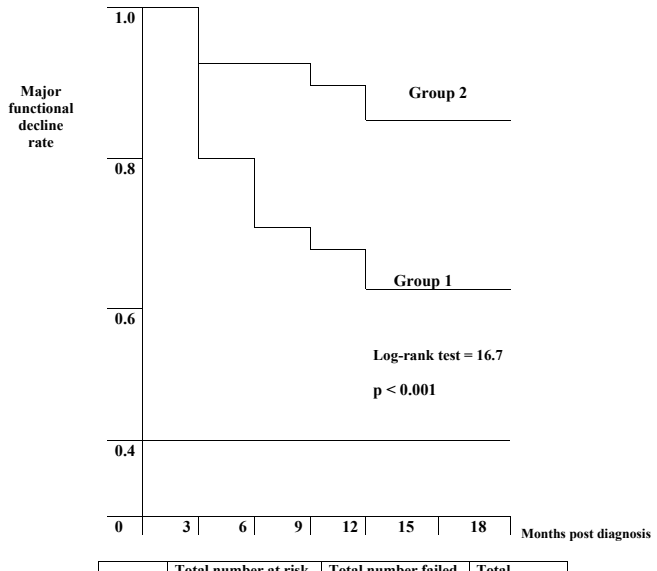
Evolution of renal-allograft function: At diagnosis of BKV-viremia, the graft function was declined significantly. The mean CCr before diagnosis, was 71.6 +/- 30.5 ml/min, decreased to 47.4 +/- 20.7 ml/min (p< 0.05) at diagnosis and it was 25 +/- 23 ml/min at the end of follow-up (Figure 4). The mean sC was respectively, 1.36 +/- 0.8 mg/dl, 1.64 +/- 1.1 mg/dl (p= 0.05) and 4.1 +/- 1.4 mg/dl (Figure 3). 32 affected recipients had previously AR episode: 26 Grade I and 6 Grade II-III according to the Banff classification. 12 (23%) affected recipients lost their graft at mean of 11 months after diagnosis of BKV-viremia. 5 of this because of developed PVAN, and other 7 affected recipients because of rejection. In according with Drachenberg et al. [18] classification, 1 affected recipient developed type B PVAN, 2 affected recipients developed a type A PVAN, and in 2 remaining affected recipients was made a presumptive-PVAN diagnosis (Table 3).

Outcome of BKV infection: The mean blood viral load decreased

from 14.2 +/- 7.5 x 10^4 copies/ml at diagnosis of BKV-viremia, to 11.8 +/- 5.6 x 10^4 copies/ml at 3 months after diagnosis ($p = 0.47$), to 8.52 +/- 4.97 x 10^4 copies/ml at 6 months after diagnosis ($p = 0.15$), to 6.5 +/- 5.3 x 10^4 copies/ml at 9 months after diagnosis ($p < 0.05$), to 3.05 +/- 4.1 x 10^4 copies/ml at 12 months after diagnosis ($p < 0.05$), and to 1.73 +/- 2.43 x 10^4 copies/ml at the end of follow-up ($p < 0.001$) (Figure 2), it became completely negative in 23 (43.3%) affected recipients. The larger clearance of BKV was observed after a mean of 9 months after MMF discontinuation. BKV urinary shedding was observed at mean onset of 3.9 +/- 1.7 months after RT. In 42 (80%) affected patients, viruria preceded viremia by 3.7 +/- 2.3 months while 11 (20%) affected patients showed concomitant detection of viruria and viremia (data not shown). The mean duration of viruria was 13.6 +/- 8.3 months and it became completely negative in 17 (32%) affected patients (Table 3).

Group 2

Evolution of graft function: At diagnosis of BKV-viremia, the graft function was declined significantly but it improved after the treatment. The mean CCr before diagnosis of BKV-viremia was 69.1 +/- 25 ml/min, it decreased to 40.6 +/- 17.5 ml/min ($p< 0.05$) at diagnosis and it was 68.5 +/- 18.1 ml/min at the end of follow-up (Figure 4). The mean sC was, respectively, 1.27 +/- 0.9 mg/dl, 1.6 +/- 1.22 mg/dl ($p< 0.05$) and 1.31 +/- 1.1 mg/dl (Figure 3). Once BKV was cleared from the blood, the graft function improved, obtaining similar values before diagnosis of BKV-viremia. At the end of follow-up the graft function was stabilized in 22 (56%) affected patients, it was improved in 8 (21%), and it was deteriorated in 9 (23%) (data not shown). All affected recipients had not new AR episodes. 4 (10%) affected recipients developed PVAN. 1 affected recipient developed a type B PVAN, 1 affected recipient developed a type A PVAN, and in remaining 2 affected recipients were made a presumptive-PVAN diagnosis. 24 (16.8%) affected recipients had previously AR episode: 20 Grade I and 4 Grade II-III according to the Banff classification (Table 3).



	Total number at risk at diagnosis	Total number failed (with event)	Total number censored
Group 1	53	19	34
Group 2	39	5	34

Figure 1: Kaplan-Meier plots for major graft functional decline (defined by graft loss or doubling of serum creatinine after the diagnosis of BKV-viremia) in either groups of patient stratified by Treatment Group. Major graft functional decline rate was significantly different: Group 2 had 13% of major graft functional decline rate at the end of follow-up while Group 1 had 36% of major graft functional decline rate ($p < 0.001$).

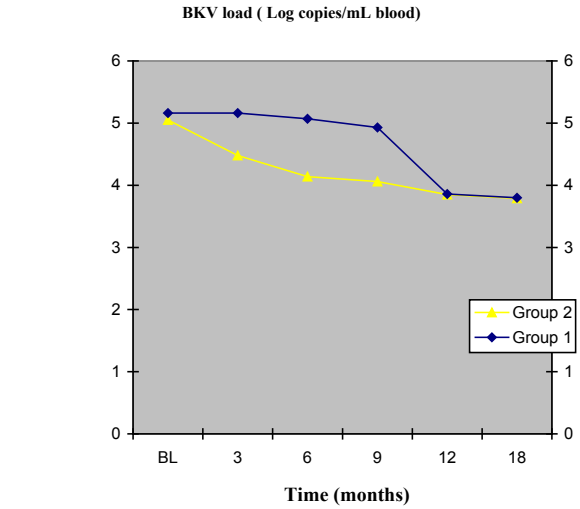


Figure 2: Evolution of the mean BKV blood viral load in two groups. In Group 2 the larger clearance of the mean blood viral load decreased significantly since the third month after therapeutic intervention. In Group 1 the larger clearance of the mean blood viral load decreased significantly since the ninth month after therapeutic intervention and became similar Group 2 since the twelfth month after therapeutic intervention. See text for details.

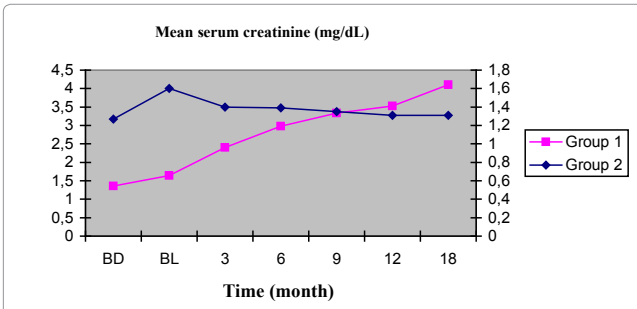


Figure 3: Evolution of the mean serum creatinine in two groups. At the time of BKV-viremia diagnosis, the median serum creatinine was increased in either groups. At the end of follow-up the median serum creatinine of Group 1was increased further on, instead in Group 2 it was decreased.

Outcome of BKV infection

The mean viral load decreased in all patients from 11.46 +/- 7.84 x 10^4 copies/ml at diagnosis of BKV-viremia to 3.06 +/- 2.83 x 10^4 copies/ml at 3 months after diagnosis ($p < 0.001$), to 1.38 +/- 0.42 x 10^4 copies/ml at 6 months after diagnosis ($p < 0.05$); to 1.14 +/- 0.49 x 10^4 copies/ml at 9 months after diagnosis ($p = 0.15$); to 0.71 +/- 0.53 x10^4 copies/ml at 12 months after diagnosis ($p = 0.10$), to 0.62 +/- 0.53 x 10^4 copies/ml at the end of follow-up ($p = 0.10$) (Figure 2). The mean duration of viruria was 7.3 +/- 4.5 months ($p < 0.05$ vs Group 1) (Table 3) and it became completely negative in 31 (80%) affected patients (data not shown). The larger clearance of BKV-viremia was observed after a median of 3 months of therapy. BKV urinary shedding was observed in all affected patients after a mean onset of 4 +/- 2.1 months from RT. In 30 (77%) affected patients, viruria preceded viremia by 2.7 +/- 1.5 months while 9 (23%) affected patients showed concomitant detection

of viruria and viremia (data not shown).

The results of the multivariate Cox regression analysis of possible risk factors between two groups for major graft functional decline with time to major graft functional decline as dependent variable are displayed in Table 4. Important risk factors are: a precedent AR episode (Hr = 3.11, 95% CI = 1.13 – 9.24, p = 0.04), a development of PVAN (Hr = 3.52, 95% CI = 1.63 – 7.52, p < 0.01), a history of CMV prophylaxis/treatment (Hr = 3.32, 95% CI = 1.51 – 7.60, p = 0.04), a maintenance immunosuppressive therapy based on Fk, MMF, Cs versus a maintenance immunosuppressive therapy based on CyA, MMF, Cs (Hr = 0.99, 95% CI = 0.97 – 1.00, p = 0.02), a shorter duration of pre-transplant dialysis (3 – 10 months versus > 10 months) (Hr = 0.59, 95% CI = 0.34 – 1.00, p = 0.05). The relative risk of graft functional decline associated with Group 2 versus Group 1 treatment, is approximately one third in Group 2 versus Group 1 (Hr = 0.38, 95% CI = 0.27 – 0.63, p <0.001). All other parameters studied were not relevant. (see Table 4 for details). Kaplan-Meier plots for estimate the probability of overall major graft functional decline after diagnosis of BKV-viremia for either groups of patient stratified by Treatment Group are displayed in Figure 1. The major graft functional decline was significantly different between two groups: Group 2 had 13% major graft functional decline rate (5 affected recipients had doubling of serum creatinine after follow-up) while Group 1 had 36% major graft functional decline rate (7 affected recipients had doubling of serum creatinine and 12 affected recipients had graft loss after follow-up) (p<0.001).

Adverse events

Two patients developed an increase of plasma calcium levels (>11 mg/dl) after two weeks of therapy. We then decided to decrease calcitriol daily dosage by 25-50 %. Three patients developed diarrhoea 5 days after beginning therapy. One patients had transient an upper body maculopapular skin rashes with pruritus. An increased of bilirubin and transaminases was observed in five patients but remained below the double level of the upper norm.

Discussion

Diagnosis of PVAN has been significantly facilitated using markers of BKV replication, but the treatment remains a major challenge [20]. In our study, the incidence of BK-viremia in 52 months was 27%, which is in the range reported by other investigators [21]. Multivariate analysis revealed that the use of a maintenance immunosuppressive therapy based on Fk, MMF and steroid is an important risk factor for BKV-viremia. The results of previous publications on therapy regimens based on Fk or CyA, as particular risk factors for PVAN, have been conflicting. In vitro data indicate that Fk is the strongest inhibitor of BKV-specific T cells [22,23]. Currently Fk and MMF generally believed to be associated with a higher incidence of PVAN [24,25,26]. It is also true that PVAN was essentially an unknown entity in the era of CyA-based immunosuppression, with increasing of its identification coinciding with inclusion of Fk and MMF in immunosuppressive regimens, and therefore we can be assumed that the higher intensity of immune suppression with Fk and MMF was decisive rather than an additional effect of a particular class of substances. This assumption remains controversial but it is supported by the association between their dosages and/or blood levels and risk of PVAN [27]. The second important risk factor identified in this study was a history of AR episode. It has been postulated that increased tubular epithelial regeneration, following tubular necrosis caused by rejection, contributes to the reactivation of BKV-infection [28]. But it is more important the increasing of cumulative immunosuppression after recurring episodes of rejection that often necessitated the use of more steroid boluses and a high dose of Fk and MMF. In fact acute tubular necrosis caused by

Transplant characteristic (reference level)	Hazard ratio	95 % CI	p
Treatment group (Group 1)	0.38	0.27 – 0.63	<0.001
Donor gender (male) Female	1.51	0.71 – 3.11	0.26
Recipient gender (male) Female	2.00	0.85 – 5.45	0.14
Recipient age at transplantation (35- 54) years > 54 years	0.72	0.33 – 1.51	0.34
Donor age (35- 54) years > 54 years	1.22	0.43 – 3.24	0.72
Renal disease (unspecified) Glomerulonephritis Interstitial nephritis Polycystic kidney disease	1.13 0.94 0.85	0.38 – 3.31 0.58 – 1.52 0.13 – 5.48	0.83 0.79 0.85
Pretransplant dialysis time (> 10 months) 3 – 10 months	0.59	0.34 – 1.00	0.05
Pretransplant dialysis type (peritoneal dialysis) Hemodialysis	2.92	0.81 – 10.51	0.11
Donor source (living donor) Deceased donor	2.21	0.83 – 5.62	0.10
AR episode BD (no episode) yes episode	3.11	1.13 – 9.24	0.04
CMV proph/treat BD (no proph/treat) yes proph/treat	3.32	1.51 – 7.60	0.04
Immunosuppressive therapy (CyA,MMF,Cs) Fk,MMF,Cs	0.99	0.97 – 1.00	0.02
Development of PVAN (no) yes	3.52	1.63 – 7.52	< 0.01

Risk factors for major graft functional decline were a precedent AR episode, a development of PVAN, a history of CMV prophylaxis/treatment before the diagnosis of BKV-viremia, a maintenance immunosuppressive therapy based on Fk, MMF, Cs versus a maintenance immunosuppressive therapy based on CyA, MMF, Cs, a major duration of pretransplant dialysis (> 10 months versus 3 – 10 months). Group 2 had approximately one third of major graft functional decline than Group 1. Fk = tacrolimus, MMF = mycophenolate mofetil, Cs = steroid, CyA = cyclosporine

e, ATG = antithymocyte globulins, CMV = cytomegalovirus, AR = acute rejection, BD = before diagnosis.

Table 4: Risk factors for major graft functional decline between two groups at the and of minimum follow-up. Multivariate Cox regression analyses with time to major graft functional decline as the dependent variable.

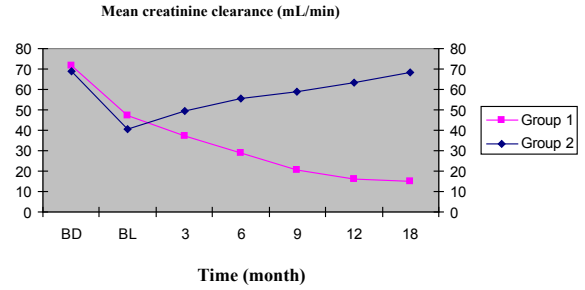


Figure 4: Evolution of the mean creatinine clearance in two groups. At the time of BKV-viremia diagnosis, the median creatinine clearance was decreased in either groups. At the end of follow-up the median creatinine clearance of Group 1 was further decreased, instead in Group 2 it was increased.

both cold and warm ischaemia is also associated with accelerated regeneration of the tubular epithelium but does not favour the development of PVAN [29]. Instead the type of treatment for AR episode (lymphocyte-depleting agents or steroid pulses) was not significantly associated with the development of BK-viremia. Moreover there was a significant association of BKV-viremia and a history of CMV prophylaxis/treatment. Other recipient and donor characteristics, such as female donor, older age recipient, older age donor, deceased donor kidneys, are also associated with a higher risk of BKV-infection, but they probably play a secondary role. Other factors were not significantly associated with episode of BKV infection. Among the risk factors contributing to BKV infection development, a central role is played by the disruption of the balance between BKV replication and virus specific immune surveillance [29,30]. The host immune response is of central importance for limiting primary viral infections and for controlling the virus carrier state [31]. Innate immunity and non specific effectors represent a first line of defence against infection, until the development of specific humoral and cellular immunity. Specific antibodies may accelerate the clearance of primary infection and contribute to protection from BK viremia, but have a minimal role in the containment of polyomavirus-related disease [32]. The development of alternative therapeutic strategies, based on control of infection with restoring-specific immune functions, is an attractive option. The results of this study seems that activated vitamin D may help in this regard. This is the first study of a small RTRs group in which was evaluated the safety and efficacy of use of fluoroquinolones and activated vitamin D combination therapy for to reduce BKV-replication compared with a protocol based on an early MMF discontinuation. The analysis of our results suggest that the clinical efficacy of this therapy is greater than an early MMF discontinuation. The mean blood viral load during follow-up decreased in both groups but the larger clearance of BKV was observed after a mean of 3 months of therapy in Group 2 and after a mean of 6 months after MMF discontinuation in Group 1. PCR-BKV became negative in 33 (85%) affected patients of Group 2 versus 23 (43.4%) affected patients of Group 1 ($p < 0.05$) indicating a more effective viral clearance facilitated by this therapy. Similar data we saw for viruria: the mean urine viral load duration was 7.3 +/- 4.5 months in Group 2 versus 13.6 +/- 8.3 months in Group 1 ($p < 0.05$), and it became completely negative in 31 (80%) affected patients of Group 2 versus 17 (32%) affected patients of Group 1 ($p < 0.05$). At the end of the minimum follow-up multivariate analysis revealed that risk factors for major graft functional decline considering time to major graft functional decline as the dependent variable were: a precedent AR episode, the development of PVAN, a history of CMV prophylaxis/treatment before the diagnosis of BKV-viremia, a maintenance immunosuppressive therapy based on CyA, MMF, Cs versus a maintenance immunosuppressive therapy based on Fk, MMF, Cs, a major duration of pretransplant dialysis (> 10 months versus 3-10 months). A thing of great moment is that the relative risk of graft functional decline associated with Group 2 versus Group 1 treatment, adjusted for other covariates, is approximately one third. We have seen extra benefits of floroquinolones and activated vitamin D combination therapy than the MMF discontinuation. In fact at the end of follow-up, the Group 2 graft survival rate was greater than Group 1. In Group 2 none affected patients had lost their graft versus 12 (23%) affected patients of Group 1. In Group 2 the graft function was stabilized in 22 (56%) affected patients, and it was improved in 8 (21%). In Group 2 none affected patients had new episodes of AR versus 7 (13%) in affected patient of Group 1. The incidence of new AR episodes in Group 1 (13%) was higher than reported in study of Brennan et al. [5] in which the rejection rate was 5% but the raising of our results directly translate to centers with historically have lower rates of AR. This patients had a previously AR steroid resistant and they developed a new episode of vascular rejection during the minimum follow-up treated unsuccessfully with ATG and the patients had lost their graft. However, administration of ATG, while augment the overall state of immunosuppression, did not result in a reactivation of BKV, as evidenced by the absence of an increased of PCR BKV during the follow-up. It is quite possible that this patients had reached the later stages of PVAN, characterized by interstitial fibrosis and tubular atrophy [32], which may reduce the amount of viable renal tissue permissive for viral replication. But it is also possible that this agent simply does not stimulate the viral replication, particularly during quiescent infection. Hirsch et al. [33] found no an association of ATG with development of BK viremia and with PVAN when they were used as induction therapy at the time of transplantation, although there was an association when they were used as treatment for AR, suggesting that allograft damage by AR and not ATG predisposed to viral reactivation. Moreover 5 (9.4%) of 12 affected patients of Group 1 that lost their graft, developed PVAN. Also 4 (10%) affected patients of Group 2 developed PVAN but none of this lost their graft during the minimum follow-up even if any degree of moderate to severe interstitial inflammation related to the presence of BK virus, normally result in a progressive graft damage and loss of function [32]. This is indirectly another point toward a potential benefit of this therapy. Fluoroquinolones and calcitriol combination therapy is well tolerated by the majority of our patients. No serious adverse events, no serious hepatic or hematologic complications were noted. Five patients had a lower increased of bilirubin and transaminases. Diarrhoea and skin rashes was observed in few patients for a short time. Two patients developed an increase of plasma calcium levels and then daily dosage was decreased by 25-50% with consequent their normalization and without a decreased of graft function. Several types of bias may have been introduced in this study.

Firstly this is a non-randomized control study and the patient number is limited. Secondly, an important work-up bias is the design of the work using sequential, historical controls that introduced possible confounding by era effects (ie, changes in case mix or other measured and unmeasured treatment factors over time) due at different times of treatment for two groups. Lastly, viral genotypic and phenotypic properties might have influenced the clinical response to therapeutic interventions but were not assessed in the current study.

Conclusion

BKV-infection continues to be an important cause of interstitial nephritis and of graft loss in RTRs. Early strategies are based upon the idea that periodic monitoring of BK viral loads allow for the diagnosis of early systemic infections. This facilitates prompt treatment, which may limit morbidity.

One significant drawback of early strategies is that success requires vigilance and adherence to monitoring protocols. A better approach to the management of BKV-infection may be to emulate strategies used to manage CMV-infection in which antimicrobial prophylaxis is commonplace for the prevention of CMV disease. Early reduction of the maintenance immunosuppression is recommended for preventing PVAN, but a progressive graft dysfunction and a graft loss occur in many cases. To our knowledge, this is the first study evaluating a pharmacologic intervention with a combination therapy based on fluoroquinolones and activated vitamin D versus an early discontinuation of MMF for the treatment of BKV-viremia after RT. The mean blood viral load

decreased in both groups but the graft function was stabilized only in Group 2. No graft loss occurred in Group 2 compared with 12 grafts loss occurred in Group 1. 5 of this were loss because of they developed PVAN and 7 were loss because of they developed a new AR episode. The relative risk of graft functional decline is approximately one third in Group 2 versus Group 1. These preliminary results are encouraging but must be confirmed by randomized study to prospectively evaluate the effect of this combination therapy on graft function, graft survival, and viral load in presence of this important complication.

Acknowledgments

The Authors thank Anna Morra for her technical assistance. No funding source was involved. No financial and personal relationships with other people or organization have influenced the work. Possible conflicts of interest following publication of this work are disclosed

References

1. Hirsch HH, Steiger J (2003) Polyomavirus BK. Lancet Infect Dis 3: 611-623.
2. Hirsch HH, Brennan DC, Drachenberg CB, Ginevri F, Gordon J, et al. (2005) Polyomavirus-associated nephropathy in renal transplantation: Interdisciplinary analyses and recommendations. Transplantation 79: 1277-1286.
3. Mayr M, Nickeleit V, Hirsch HH, Dickenmann M, Mihatsch MJ, et al. (2001) Polyomavirus BK nephropathy in a kidney transplant recipient: critical issues of diagnosis and management. Am J Kidney Dis 38: 13.
4. Nickeleit V, Klimkait T, Binet IF, Dalquen P, Del Zenero V, et al. (2000) Testing for polyomavirus type BK DNA in plasma to identify renal-allograft recipients with viral nephropathy. N Engl J Med 342: 1309-1315.
5. Brennan DC, Agha I, Bohl DL, Schnitzler MA, Hardinger KL, et al. (2005) Incidence of BK with tacrolimus versus cyclosporine and impact of preemptive immunosuppression reduction. Am J transplant 5: 582-594.
6. Ramos E, Drachenberg CB, Wali R, Hirsch HH (2009) The decade of polyomavirus BK-associated nephropathy: state of affairs. Transplantation 87: 621-630.
7. Nickeleit V, Mihatsch MJ (2006) Polyomavirus nephropathy in native kidneys and renal allografts: an update on an escalating threat. Transpl Int 19: 960-973.
8. Ginevri F, Azzi A, Hirsch HH, Basso S, Fontana I, et al. (2007) Prospective monitoring of polyomavirus BK replication and impact of pre-emptive intervention in pediatric kidney recipients. Am J Transplant 7: 2727-2735.
9. Ali SH, Chandraker A, De Caprio JA (2007) Inhibition of Simian virus 40 large T antigen helicase activity by fluoroquinolones. Antivir Ther 12: 1-6.
10. Chandraker A, Ali S, Drachenberg CB, Wali R (2004) Use of fluoroquinolones to treat BK infection in renal transplant recipients. Am J Transplant 4: 587.
11. Leung AY, Chan MT, Yung KY, Cheng VC, Chan KH, et al. (2005) Ciprofloxacin decreased polyoma BK virus load in patients who underwent allogenic hematopoietic stem cell transplantation. Clin Infect Dis 40: 528-537.
12. Gabardi S, Waikar SS, Martin S, Roberts K, Chen J, et al. (2010) Evaluation of fluoroquinolones for the prevention of BK viremia after renal transplantation. Clin J Am Soc Nephrol 5: 1298-1304.
13. Liu PT, Stenger S, Li H, Wenzel L, Tan BH, et al. (2006) Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science 311: 1770-1773.
14. Selvaraj P (2011) Vitamin D, Vitamin D receptor, and cathelicidin in the treatment of tuberculosis. Vitam Horm 86: 307-325.
15. Gal-Tanamy M, Bachmetov L, Ravid A, Koren R, Erman A, et al. (2011) Vitamin D: an innate antiviral agent suppressing Hepatitis C virus in human hepatocytes. Hepatology 54: 1570-1579.
16. Aloia JF, Li-Ng M (2007) Re: epidemic influenza and vitamin D. Epidemiol Infect 135: 1095-1098.
17. Kasiske BL, Vazquez MA, Harmon WE, Browns RS, Danovitch GM, et al. Recommendations for the outpatients surveillance of renal transplant recipients. J Am Soc Nephrol 11: S1-S86.
18. Drachenberg CB, Papadimitriou JC, Hirsch HH, Wali R, Crowder C, et al. (2004) Histological patterns of Polyomavirus nephropathy: correlation with graft outcome and viral load. Transplant 4: 2082-2092.
19. Racusen LC, Solez K, Colvin RB, Bonsib SM, Castro MC, et al. (1999) The Banff 97 working classification of renal allograft Pathology. Kidney Int 55: 713-723.
20. Almeras C, Foulogne V, Garrigue V, Szwarc I, Vetromile F, et al. (2008) Does reduction in immunosuppression in viremic patients prevent BK virus nephropathy in de novo renal transplant recipients?. A prospective study. Transplantation 85: 1099-1104.
21. Razonable RR, Brown RA, Humar A, Covington E, Alecock E, et al. (2005) A longitudinal molecular surveillance study of human polyomavirus viremia in heart, kidney, liver, and pancreas transplant patients. J Infect Dis 192: 1349-1354.
22. Vasudev B, Hariharan S, Hussain SA, Zhu YR, Bresnahan BA, et al. (2005) BK virus nephritis: risk factors, timing, and outcome in renal transplant recipients. Kidney Int 68: 1834-1839.
23. Egli A, Kohli S, Dickenmann M, Hirsch HH (2009) Inhibition of polyomavirus BK-specific T Cell responses by immunosuppressive drugs. Transplantation 88: 1161-1168.
24. Barri YM, Ahmad I, Ketel BL, Barone GW, Walker PD, et al. (2001) Polyoma viral infection in renal transplantation: the role of immunosuppressive therapy. Clin Transplant 15: 240-246.
25. Mengel M, Marwedel M, Radermacher J, Eden G, Schwarz A, et al. (2003) Incidence of Polyomavirus-nephropathy in renal allografts: influence of modern immunosuppressive drugs. Nephrol Dial Transplant 18: 1190-1196.
26. Chen Y, Trofe J, Gordon J, Du Pasquier RA, Roy-Chaudhury P, et al. (2006) Interplay of cellular and humoral immune responses against Bk virus in kidney transplant recipients with polyomavirus nephropathy. J Virol 80: 3495-3505.
27. Mengel M, Marwedel M, Radermacher J, Eden G, Schwarz A, et al. (2003) Incidence of Polyomavirus-nephropathy in renal allografts: influence of modern immunosuppressive drugs. Nephrol Dial Transplant 18: 1190-1196.
28. Nickeleit V, Hirsch HH, Zeiler M, Gudat F, Prince O, et al. (2000) BK-virus nephropathy in renal transplants-tubular necrosis, MHC-class II expression and rejection in a puzzling game. Nephrol Dial Transplant 15: 324-332.
29. Schold JD, Rehman S, Kayle LK, Magliocca J, Srinivas TR, et al. (2009) Treatment for BK virus: incidence, risk factors and outcomes for kidney transplant recipients in the United States. Transpl Int 22: 626-634.
30. Comoli P, Azzi A, Maccario R, Basso S, Botti G, et al. (2004) Polyomavirus BK-specific immunity after kidney transplantation. Transplantation 78: 1229-1232.
31. Binggeli S, Egli A, Schaub S, Binet I, Mayr M, et al. (2007) Polyomavirus BK-specific cellular immune response to VP1 and large T-antigen in kidney transplant recipients. Am J Transplant 7: 1131-1139.
32. Trofe J, Hirsch HH, Ramos E (2006) Polyomavirus-associated nephropathy: Update of clinical management in kidney transplant patients. Transpl Infect Dis 8:76-85.
33. Hirsch HH, Knowles W, Dickenmann M, Passweg J, Klimkait T, et al. (2002) Prospective study of polyomavirus type BK Replication and nephropathy in renal transplant recipients. N Engl J Med 347: 488-496.