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LONG-TERM OUTCOME OF SCREENING FOR POLYOMA BK VIRUS INFECTION IN KIDNEY TRANSPLANT RECIPIENTS

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*BK virus (BKV) infection was studied prospectively in 50 unselected consecutive patients who had undergone kidney transplantation. Infection was monitored for one year after transplantation. Viral DNA in urine (viruria) and plasma (viremia) samples was detected by nested, qualitative polymerase chain reaction. BKV screening data was available for 92% (n = 46) of patients enrolled in the study. Four groups of patients were distinguished: uninfected patients (group 1, n = 30), patients with viruria (group 2, n = 3), patients with viremia (group 3, n = 6) and patients with developed BKV nephropathy (group 4, n = 7). Infection was observed starting form the first month, and the maximum number of patients with active BKV infection occurred at six months after transplantation. Five-year graft survival was 69% for patients with any evidence of BKV infection, compared with 80.0% (*P = NS*) for patients without BKV infection. The best graft function was observed in group one patient (mean serum creatinine 130 mkmol/l and glomerular filtration rate (GFR) 60.9 ml/min) and the worst in group 4 (mean serum creatinine 180 mkmol/l and GFR 52.31 ml/min) at five years after transplantation. Five-year patient survival was 82.6% and was not affected by presence of BKV infection.*

Key words: *kidney transplantation, polyoma BK virus, graft survival.*

INTRODUCTION

Primary infection with polyoma BK virus (BKV) mostly occurs during childhood. Usually it is asymptomatic and adult seroprevalence is 60–90% in the general population (Knowles *et al*., 2003; Egli *et al*., 2009). After primary infection, the virus persists in a latent state in target cells, mainly epithelial cells in the kidney and urinary tract (Chesters *et al*., 1983). Virus reactivation can occur as viruria and/or viremia. In healthy adults virus reactivation is intermittent and in rare cases can be detected as low grade viruria (Polo *et al*., 2004). In most cases an active BKV infection develops within a year after kidney transplantation (Hirsch *et al*., 2002; Vasudev *et al*., 2005). In 30–50% kidney transplant recipients viruria progresses to viremia (Bonvoisin *et al*., 2008). An active BKV virus infection can cause lesion in the graft known as polyoma BK virus nephropathy (BKVN) (Hirsch *et al*., 2002). BKVN causes progression of graft dysfunction and can lead to premature graft loss (Ramos *et al.,* 2002). It affects 1–10% of renal transplant recipients, and has become more widespread since 1995 due to more potent immunosuppression availability (Binet *et al*., 1999). Therefore, the mainstay of therapy for polyoma BKV nephropathy is to reduce or discontinue immunosuppressive drugs (Comoli *et al*., 2006). As no anti-BKV drugs exist and no evidence-based recommendations can be made for the treatment of active BKV infection (Hirsch *et al*., 2009), each transplantation centre has its own strategy. The aim of this paper is to summarize our experience in the treatment of active BKV infection.

MATERIALS AND METHODS

Study cohort. The study was performed in the Latvian Transplantation Centre of Pauls Stradiņš Clinical University Hospital in cooperation with the Institute of Pathology and Department of Oncovirology of the Augusts Kirhenšteins Institute of Microbiology and Virology in Rīga Stradiņš University. The prospective study included 50 renal transplant recipients (mean age 46 ± 13.8 years, 52% males) who underwent deceased donor transplantation in 2007. That kidney transplantation was the first for 41 (82%) patients and the second for 9 (18%) patients. All patients with two consecutive positive BK viremia measurements or patients who developed an unexplained elevation of serum creatinine underwent a kidney graft biopsy to evaluate BKVN. Acute rejection was defined by Banff criteria (Sis *et al*., 2010). The study was approved by the Ethics Committee of Rīga Stradiņš University.

Immunosuppression. All patients included in this study received induction immunosuppression during kidney allografting surgery, and included an agent that contained anti-CD25 monoclonal antibody, i.e., basiliximab 20 mg intrave-

nously on days 0 and 4, daclizumab 1 mg/kg intravenously on days 0 and 15, or the polyclonal antibody antilymphocyte globulin (ATG) 1.5–3 mg/kg intravenously on the first 3–5 post-transplant days. The initial maintenance immunosuppression in all patients consisted of methylprednisolone given intravenously in doses of 500 mg on the day of the surgery, gradually over five days, followed by doses of oral prednisone starting from 0.5 mg/kg per day and gradually reduced such that the average dose after a month was 20 mg per day and that after six months was 5–10 mg per day. Calcineurin inhibitors and antiproliferative drugs were given on the first postoperative day. Two antifroliferative drugs were used: mycophenolate mofetil (MMF) at initial average dose 2 g per day orally, or azathioprine (AZA) at initial dose 100–150 mg per day orally adjusted per white blood cells count. Two calcineurin inhibitors were used: cyclosporine A (CSA) at initial average dose 3–4 mg/kg per day orally adjusted to a trough level in blood of 150–200 ng/ml during the first 3 post-transplant months and 100–200 ng/ml subsequently. The other calcineurin inhibitor, which was received by some patients after treatment of severe acute rejection, was tacrolimus (TAC). Tacrolimus was initiated at 0.1 mg/kg per day oral, aiming whole blood trough level of 5–10 ng/ml during the first three months and 4–8 ng/ml subsequently. Patients who developed side effects of calcineurin inhibitors received sirolimus at initial dose 2 mg per day orally, targeting a trough blood level of 5–10 ng/ml. All patients experiencing an acute rejection episode as proved by biopsy were treated with 3–5 boluses of intravenous methylprednisolone. Steroid-resistant rejections were treated with ATG (1.5–3 mg/kg per day intravenously over 10–14 days).

Diagnostics of BKV infection. During the study, virus infections diagnostics was performed before kidney transplantation, two weeks after surgery and further every three months during the first post-transplant year, i.e., on the 3^{rd}, 6^{th}, 9^{th} and 12^{th} post-transplant month. Blood from the peripheral vein was collected in a blood tube with anticoagulant (EDTA), and a middle portion of the morning urine flow was collected in a sterile urine container. Qualitative nested polymerase chain reaction (PCR) testing was performed to detect BKV infection. Markers of the active viral infection were viral sequences in the DNA isolated from the cell-free plasma (viremia) or urine (viruria).

Management of BKV infection. Patients diagnosed for BKV in two consecutive measurements and/ or developing viral cytopathic changes characteristic for BKVN were assumed to have their immunosuppression reduced., The currently available guidelines about immunosuppression reduction schemes had not ben published yet in the time period of the study (2007 to 2008). For that reason, immunosuppression was reduced with caution. The MMF dose was reduced by 500 mg per day or MMF was switched to azathioprine, or the cyclosporine dose was reduced by 50 mg per day, or the tacrolimus dose was reduced by 1–2 mg per day.

Statistics. Data are presented as means ± SD or counts and percentages, as appropriate. The groups were compared using the Student's t test, Fisher's exact test or chi-square, as needed. A P value less than 0.05 was considered statistically significant. The incidences of graft and patient survival were calculated for one-, two- and three-year periods after transplantation using the Kaplan-Meier surveillance test. The Log Rank (Mantel-Cox) test was used to compare two groups. The data were censored at the moment the patients reached the end of study period. The graft function was determined by the estimated glomerular filtration rate (eGFR) calculated by the Cockroft-Gault formula.

RESULTS

Diagnostics of the polyoma BK virus was performed in 46 of 50 patients included in the study. Two patients had died within the first three months of study (one due to acute pancreatitis with consecutive colon perforation and acute peritonitis, the other due to pulmonary artery thromboembolism), one patient had a primary non-functioning graft and another patient underwent transplantectomy due to renal artery thrombosis.

Polyoma BK virus infection was diagnosed in 16 patients. PCR testing showed BK viruria in 16 (35%) patients and BK viremia in 13 (28%) of 46 patients. BK viruria and BK viremia were not always diagnosed at the same time. In most cases, BK viruria was detected first, and viruria and viremia during the next examination. After therapy modification BK viremia disappeared first and viruria later. Three patients had only BK viruria without consecutive development of BK viremia, and 13 patients had both BK viruria and viremia. Seven (53%) of 13 patients with BK viremia developed BKV nephropathy. The diagnosis of BKV nephropathy was confirmed by characteristic cytopathic changes found on kidney graft biopsy. The other five patients recovered from BK viremia and after five years still had a functioning graft; one patient with a functioning graft died due to acute myocardial infarction 58 months after transplantation. All three patients with BK viruria recovered from it and had a functioning graft within five years after transplantation. Five (71%) of seven patients with BKV nephropathy lost their grafts within five years after transplantation. One patient, who lost his graft due to polyoma BKV nephropathy, has received a second transplant 59 months after primary surgery. The second transplant had good function and there were no signs of repeated polyoma BKV infection until the end of the study.

The 46 patients to whom screening for polyoma BK virus was completed by the end of the first post-transplant year were divided into four groups: group 1 (n = 30) – BKV infection was not diagnosed, group 2 (n = 3) — BKV viruria was diagnosed, group 3 (n = 6) — BKV viruria and viremia were diagnosed and group 4 (n = 7) — BKV viruria, viremia and BKV nephropathy were diagnosed. BKVN did not develop in the first three months of the study, but six months later it was diagnosed in four (9%)

patients who previously had viruria and viremia. On the ninth month new BKVN cases were not diagnosed. After twelve months BKVN had developed in three (6%) more patients.

Patient survival. Two of 50 (4%) patients died during the first three post-transplant months (one due to acute pancreatitis with consecutive colon perforation and acute peritonitis, the other due to pulmonary artery thromboembolism). Two patients lost their grafts during the first three study months (one patient had a primary non-function graft and the other — renal arterial thrombosis, infarctions and necrosis with subsequent transplantectomy) and these were not included for further monitoring and continued hemodialysis. All 46 patients who were monitored for BKV infection during the study were alive at the end of the first study year. One-year patient survival was 96% (Fig. 1).

One year later three (6%) patients had died and 43 patients were alive. The causes of death were: melanoma with multiple metastases, hepatic cirrhosis and coronary heart disease with acute myocardial infarction. Only one of the deceased patients (cause of the death — acute myocardial infarction) was previously diagnosed for BK viremia and BKVN. The two-year patient survival was 90%.

Overall five-year patient survival was 82.6%. During the third to fifth post-transplant years 4 (8%) patients died. The causes of death were acute myocardial infarction, hypertensive crisis and for two patients the cause of death was not known. The patient who died due to acute myocardial infarction had previously diagnosed BK viruria and viremia, the others did not have active BK virus infection. Therefore, five-year patient survival was 81.3% in the patient group with BKV infection, and 83.3% in the patient group without BKV infection. The death of patients was not related to either to BK viruria or BK viremia or BK virus nephropathy. In summary, five-year patient survival was 100% in group 2, 83.3% in group 1 similar to group 3 and 71.4% in group 4 (P = NS).

Graft survival. At the end of the first year, 46 of 50 patients had a functioning kidney graft and thus one-year graft survival was 92% (Fig. 2). Six grafts were lost during the

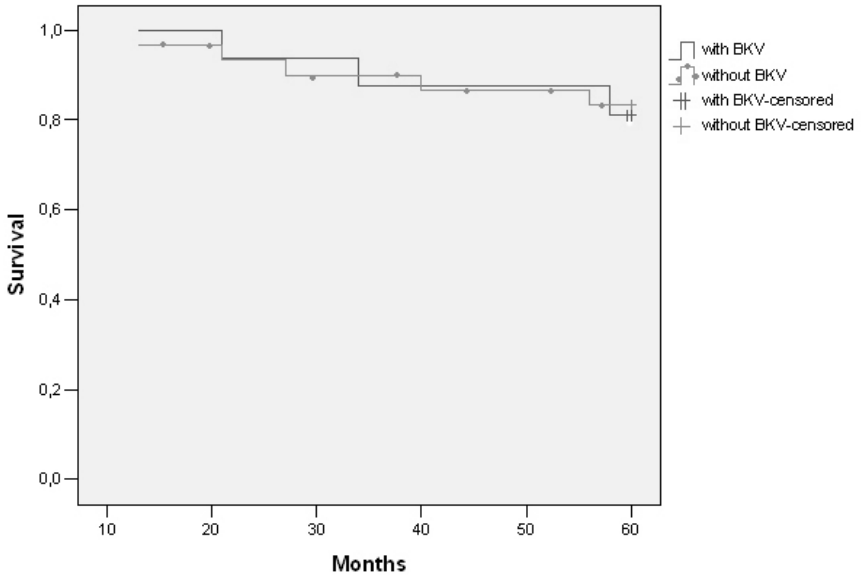

Fig. 1. Patient survival after kidney transplantation. BKV, BK virus.

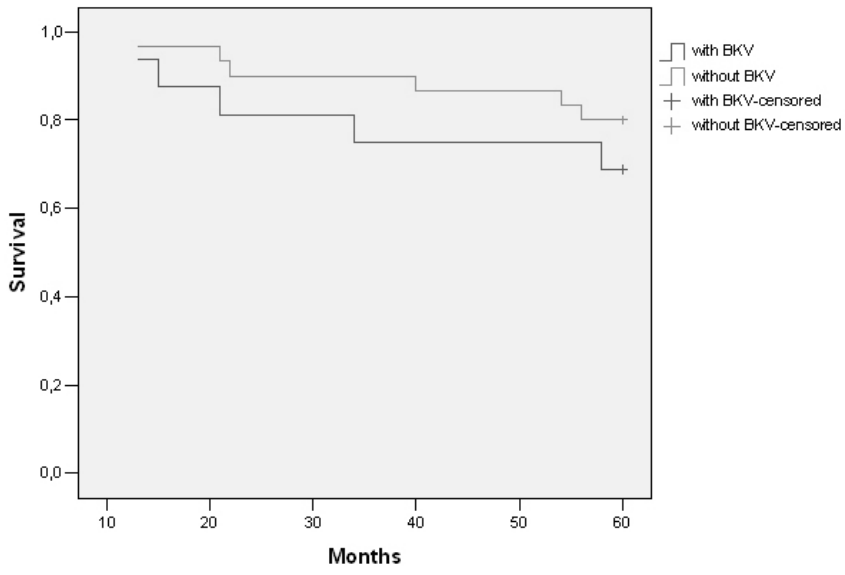

Fig. 2. Graft survival after kidney transplantation. BKV, BK virus.

second year, two of them due to BKVN, one due to acute rejection and chronic allograft nephropathy and three due to patient death. In addition, one patients who was previously diagnosed for BKVN. Thus, after two years, grafts did not function in three of seven patients with BKVN and in three of 39 in patients without BKVN (42% *vs* 7%, P <0.01). Overall 2-year graft survival was 80%. Two-year graft survival was 75% for patients with active BKV infection and, 82% (P = NS) for patients without active BKV infection.

Overall five-year graft survival was 76.1% for patients screened for BKV infection. Graft survival was 68.8 % in patients with active BKV infection and 80% (P = NS) in patients without BKV infection. Five more grafts had been lost by five years after transplantation. The cause of graft loss was death of a patient with a functioning graft in four cases, one of whom had been previously diagnosed for BKVN. One graft was lost due to repeated urinary tract infections and chronic allograft nephropathy. Five-year graft survival was 82.1% for patients without developed BKVN and 42.9% for patients with diagnosed BKVN (P < 0.01). Five-year graft survival was 80% in group 1, 100% in group 2, 83.3% in group 3 and 42.9% in group 4 (P < 0.01).

Rejection. Acute rejection occurred in 22 (48%) of 46 patients by five years after transplantation. Fifteen (33%) patients experienced acute rejection in the first year after transplantation and seven (15%) patients suffered acute rejection during the subsequent 2–5 years. Banff-grade I rejections occurred in 16 (35%) patients, grade II rejections in 4 (9%) patients and two (4.5%) patients had grade III acute rejection. In nine cases rejection was steroid resistant and was treated with ATG. In four of 15 patients who experienced acute rejection, active BKV infection (P < 0.05) appeared later. One of those 15 patients developed BKVN at the end of first post-transplant year (P = NS). However, the grade of acute rejection was not related to reactivation rate of BKV infection. The development of active BKV infection or BKVN was not associated with treatment of rejection with ATG year (P = NS).

Graft function. Mean serum creatinine was 140 ± 8 mkmol/l and GFR level was 63.52 ± 3,19 ml/min one year after transplantation. At the end of second year mean serum creatinine was 130 ± 5 mkmol/l and GFR was 67.6 ± 2.9 ml/min for patients with a functioning graft.

The overall mean five-year serum creatinine level was 140 ± 40 mkmol/l and the mean five-year GFR was 57.6 ± 17.0 ml/min in patients with a functioning graft. Five years after transplantation, patients without BKV infection had a significantly lower serum creatinine level (130 ± 30 mkmol/l vs 160 ± 50 mkmol/l, P < 0.05) and better GFR (60.9 ± 17.0 ml/min vs 52.1 ± 16.6 ml/min, P = NS) compared with patients who had previous active BKV infection.

Influence of reduction of immunosuppression in patients with BKV infection. No BKV-specific reagents have yet been developed. Currently, the more commonly used and accepted strategy for first-line treatment of active BKV infection and BKVN is reduction of immunosuppression, but schedules vary from study to study.

At the time of transplant all patients were prescribed prednisolone. After one year, prednisolone was discontinued in three patients with active BKV infection and in eight patients without active BKV infection. Five years after transplantation 21 patients still received prednisolone; of those 14 were in group 1, two in group 2, three in group 3 and two in group 4.

At the time of transplantation, all patients were prescribed MMF. After one year, for two patients (one due to CMV infection, another due to BKV nephropathy) treatment was switched to AZA and for two antimetabolite was discontinued (both due to gastrointestinal side effects and one of them with BK viremia and viruria). Thirty four of 35 patients with a functioning graft still received MMF at five years after transplantation and only one patient with previous BKVN had AZA.

At the time of transplantation all patients were prescribed CSA. Within the first post-transplant month treatment for two patients was switched to TAC due to severe rejection and later BKVN developed in one of them. During the next month one more patient had treatment switched to TAC and BKVN developed in this case. At the end of the first year, CSA was given to 13 patients with active BKV infection and 27 without active BKV infection (P = NS). BKVN developed in two patients with TAC and five patients with CSA in maintenance immunosuppression. Twenty five of 35 patients with a functioning graft still received CSA by the five years after transplantation and two of them had suffered BKVN. Five of 35 patients received TAC and one of them had previous BKVN.

Sirolimus was prescribed to four patients, all without previous BKV infection at five years after transplant.

After reduction of immunosuppression, BK viremia continued in six of 13 patients and viruria in all three patients. At the end of the first post-transplant year BK viremia still persisted in five of seven patients with BKVN and in four cases grafts were lost — two due to BKVN and two due to patient death.

DISCUSSION

This study demonstrated a good long-term graft function, but only for grafts what were not lost up to that time. Only 70% of recipients initially included in the study had functioning grafts five years after transplantation. A similar five-year graft survival rate (72% for non-expanded criteria donors and 57% for expanded criteria donors) was reported as outcome of transplants performed in the United States between 1999 and 2009 (Axelrod *et al*., 2010).

There are several findings from our study. Firstly, the rate of active BKV was 35% and incidence of BKVN was 15% one year after kidney transplantation surgery in our centre

in the year 2007. These results are compatible with reports from other centres (Dharnidharka *et al*., 2009; Koukoulaki *et al*., 2009), but the incidence is quite high, probably due to more vigilant detection of BKV infection and usage of induction immunosuppression in all cases. Active BKV infection in the first year after transplantation was related to worse graft function subsequently. Secondly, the study identified that patients with any grade of acute rejection are at risk for later reactivation of BKV infection. This was confirmed by analysis of the role of specific immunosuppressive agents and lack of a significant relationship with development of active BKV infection. The degree of immunosuppression was sufficiently high and minimisation of immunosuppression upon detection of BK viremia was safe. In the majority of patients no acute rejection developed afterwards, except in one patient. Thirdly, we observed that, when reduction in immunosuppression was made as required, viruria continued in all patients, and viremia resumed in half of patients in a subsequent 3 to 6 month period. Finally, the results demonstrate significant graft loss in the patient group with persistent viremia and developed BKVN. Thus, we can conclude that BKVN is nonreversible and immunosuppression reduction should be started when active BKV infection is diagnosed. The study has several limitations, including absence of quantitative virus DNA detection and lack of prolonged BKV detection beyond the first post-transplant year.

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POLIOMAS BK VĪRUSA SKRĪNINGA ATTĀLIE REZULTĀTI PACIENTIEM AR NIERES TRANSPLANTĀTU

Poliomas BK vīrusa infekcija tika prospektīvi diagnosticēta 50 pacientiem, kam nieres transplantācija bija veikta 2007. gadā. Vīrusa genomu secību klātbūtnes konstatēšanai asins plazmas DNS (virēmija) un urīna DNS (virūrija) tika izmantota polimerāzes ķēdes reakcijas metode ar iekšējo praimēšanu. Vīrusa genoma secību skrīninga rezultāti bija pieejami 92% (n = 46) no pētījumā iesaistītajiem pacientiem. Ņemot to vērā, pacienti tika iedalīti četrās grupās: ar poliomas BK vīrusu neinficēti pacienti (1. grupa, n = 30), pacienti ar virūriju (2. grupa, n = 3), pacienti ar virēmiju (3. grupa, n = 6) un pacienti, kuriem attīstījās BK vīrusa nefropātija (4. grupa, n = 7). Aktīva poliomas BK vīrusa infekcija trim pacientiem tika diagnosticēta jau pirmā pēctransplantācijas mēneša laikā, bet visvairāk pacientu (n = 8) ar aktīvu poliomas BK vīrusa infekciju bija sešus mēnešus pēc operācijas. Nieres transplantātu piecu gadu dzīvildze pacientiem ar virūriju un/vai virēmiju bija 69%, bet pacientiem bez infekcijas — 80% (P = NS). Vislabākā transplantātu funkcija piecus gadus pēc operācijas bija pacientiem 1. grupā (vidējais seruma kreatinīns 130 mkmol/l un glomerulārās filtrācijas ātrums (GFĀ) 60,9 ml/min) un vissliktākā — 4. grupā (vidējais seruma kreatinīns 180 mkmol/l un GFĀ 52,31 ml/min). Pacientu piecu gadu dzīvildze bija 82,6%, un tā nebija atšķirīga pacientiem ar vai bez poliomas BK vīrusa infekcijas.