RIGA STRADINS UNIVERSITY

Ieva Ziedina

POLYOMA BK VIRUS INFECTION IN RENAL TRANSPLANT RECIPIENTS

(SPECIALITY – NEPHROLOGY)

Summary of Doctoral Thesis

Scientific supervisors:
Habilitated Doctor of Medicine, Professor R. Rozentals
Habilitated Doctor of Biology S. Chapenko

Scientific advisors:
Doctor of Medicine, Assoc. Professor I. Folkmane
Doctor of Medicine A. Krumina

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The dissertation was performed in:
Riga Stradins University, Transplantation center of Latvia of P. Stradins Clinical University Hospital in cooperation with the Cytology Laboratory, Institute of Pathology and Department of Oncovirology of August Kirchenstein Institute of Microbiology and Virology in Riga Stradins University.

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Scientific supervisors:
Dr. habil. med., prof. R. Rozentals
Dr. habil. biol. S. Chapenko

Scientific advisors:
Dr. med., assoc. prof. I. Folkmane
Dr. med. A. Krumina

Official reviewers:
Dr. habil. med., prof. D. Gardovska, Riga Stradins University
Dr. habil. biol., prof. P. Pumpens, University of Latvia
Dr. med. T. Rainiene, Vilnus University Hospital Santariškiu klinikos

Meeting of Riga Stradins University Doctoral Board in internal medicine disciplines will take place on 13th of June, 2011, at 15.00; address: Riga Stradins University, 16 Dzirciema str., Riga.

Board secretary
Prof. M.Eglīte

INTRODUCTION

Kidney transplantation (KT) is considered to be the most efficient method for treating end stage renal disease. There has not been a noticeable improvement in long-term graft survival of cadaveric donors in the last decade, although there are significant improvements in transplantology. The graft function deterioration and loss can be determined by both immunologic and non-immunologic factors. The usage of even more stronger immunosuppressive agents shows a significant decrease in acute rejection in the last decade, but the frequency of infectious complications after kidney transplantation has increased. One of such infection which is becoming more frequent is polyoma BK virus (BKV) infection.

Primary infection with BKV mostly occurs during childhood. Usually it is asymptomatic and an adult seroprevalence is 60 – 90% in general population. After primary infection the virus persists in latent state in target cells, mainly epithelial cells in the kidney and urinary tract. In the most cases an active BKV infection develops after kidney transplantation by reactivation of latent persistent BKV infection or by primarily infecting the recipient with the transplanted organ. An active BKV virus infection can causes lesion in graft known as polyoma BK virus nephropathy (BKVN). BKVN is one of infectious complications which is becoming more frequent. Due to it the treatment of the patient after kidney transplantation is more difficult because it causes progressing graft function deterioration. It affects 1 – 10% renal transplant recipients and approximately for a half of them there are caused irreversible changes which lead to a premature graft loss. In order to prevent the development of BKVN there must be considered an issue about an active BKV infection diagnostics and screening – type and frequency of examinations. Furthermore the screening schemes can differ among the transplantation centers due to the used immunosuppressive schemes, as well as availability and costs of examinations. There is still a discussion about a more optimal BKVN therapy. It has been proved that if the treatment of BKVN is commenced in an earlier stage, the results are better and the BKVN can possibly regress. However the efficiency of the used antiviral drugs is questionable. Reducing immunosuppressive drugs is the current mainstay of therapy, but there is no agreement about the best strategy how to do it.

In order to improve the kidney transplantation results for patients having polyoma BK virus infection, we found it necessary to perform a study about the development of polyoma BK virus infection and nephropathy related to it.

STUDY ACTUALITY

In the last decade after introduction of stronger immunosuppressive agents the loss of grafts has been determined by infectious complications and patients' death due to cardiovascular or malignant complications with a functioning

graft. A more efficient prevention of the infection is becoming one of the main targets in transplantology.

In the Latvian Transplantation Centre are performed 70 – 79 kidney transplantations per year (average 31 kidney transplantation per million populations). There is used the latest immunosuppressive medication like in other EU countries, and the doctors working in the centre face the complications caused by infectious diseases every day. The previously common cytomegalovirus infection has become less frequent, because, since 2006 valganciclovir has been included in the list of medications compensated by the State. However there have not been made any studies about the frequency of the BKV infection and its complications for patients after kidney transplantation yet. In view of the successful cooperation in the previous years with the Department of Oncovirology of August Kirchenstein Institute of Microbiology and Virology concerning the study of β-herpesviruses and indications that β-herpesviruses can provoke development of other infections with their immunomodulating effect, it was decided to monitor β-herpesviruses infections and evaluate their impact on transplantation results regarding BKV infection also within this study.

The human herpesvirus subfamily β-herpesviruses includes cytomegalovirus (CMV), human herpesvirus 6 (HVV-6) and human herpesvirus 7 (HHV-7). These herpesviruses reactivate often after kidney transplantation and can occur in different ways – from a fever to direct organ impairment. The impact of CMV on the graft is known, but the impact of HHV-6 and HHV-7 in the development of complications is still being studied.

By planning the prospective study in year 2007 it was decided to perform a retrospective analysis concerning renal transplant recipients of the past two years first. During the first year after transplantation these recipients quite often, on the average of 15% cases, faced hydronephrosis in the graft due to the ureteral strictures and experienced quite early development of chronic allograft nephropathy. Those look like clinical manifestations of active BKV infection, leading to the assumption that the BKV infection could be a significant complication for renal transplant recipients in Latvia.

AIM, OBJECTIVES AND HYPOTHESES OF THE STUDY

Aim of the study

to assess the development of polyoma BK virus infection and nephropathy related to it in order to improve outcome of kidney transplantation.

Objectives of the study

1. To determine frequency of an active BK virus infection by comparing different methods of diagnostics.
2. To determine frequency of BK virus nephropathy, risk factors and impact on kidney transplantation results.
3. To analyze the interaction between BK virus and β-herpesviruses (CMV, HHV-6 and HHV-7) in renal transplant donors and recipients and their impact on kidney transplantation results.
4. To analyze the impact of the immunosuppressive therapy on BK virus activation and development of BK virus nephropathy.
5. To compare transplantation results of patients concerning which the BK virus and β-herpesviruseses infection was and was not constantly monitored.

Hypotheses of the study

1. An active BK virus infection is a complication of kidney transplantation which contributes to the development of BK virus nephropathy and graft function deterioration, as well as causes graft loss.
2. An active β-herpesvirus infection contributes to reactivation of BK virus infection.
3. A potent maintenance immunosuppressive therapy contributes to reactivation of BK virus and development of BK virus nephropathy.
4. Monitoring and therapy of active BK virus infection helps to preserve graft function and improves the quality of life of the recipients.

NOVELTY OF THE THESIS

1. At first there was analyzed the frequency of the co-infection of active polyoma BK virus and β-herpesviruses in *de novo* renal transplant recipients.

2. There was discovered an interaction between BK virus and β-herpesviruseses and diagnosed a possibly new indirect effect of β-herpesviruseses – contribution to reactivation of polyoma BK virus.

3. There was determined the impact of the co-infection of polyoma BK virus and β-herpesviruses on kidney transplantation results thus concluding that such combination of virus infections has a negative impact on the graft

function in the second half-year after transplantation and that a co-infection of HHV-6 and BKV even contributes to graft loss.

4. It was recommended to use the term "maldinātājšūnas" in the medical vocabulary in Latvian language in order to denote epithelial cells with increased nuclei which contain large ground-glass-type intranuclear polyoma BK virus inclusions.

MATERIALS AND METHODS

The study was performed in Riga Stradins University, Latvian Transplantation Centre of P. Stradins Clinical University Hospital in cooperation with the Cytology Laboratory, Institute of Pathology and Department of Oncovirology of August Kirchenstein Institute of Microbiology and Virology in Riga Stradins University.

Study population

The study included overall 181 patient after kidney transplantation. The study consisted from the retrospective and the prospective part.

The retrospective part included an analysis of 131 renal transplant recipient which underwent deceased donor transplantation in year 2005 and 2006 (Table 1).

Table 1. Details of the patients in the retrospective group

Patients	Index
Number of patients (n)	131
Age (years)	46.4 ± 12.1 (18 – 75)
Gender (males/ females)	71/60 (54.2 % / 45.8 %)
Body mass index (kg/m²)	24.6 ± 4.5
Basic diagnosis:	
Chronic glomerulonephritis	56 (40.9 %)
Chronic interstitial nephritis	20 (15.6 %)
Diabetic nephropathy	18 (13.1 %)
Polycistic kidney disease	21 (15.3 %)
Hypertensive nephropathy	8 (5.8 %)
Lupus nephritis	4 (2.9 %)
Unknown origin chronic kidney disease	6 (4.2 %)
Kidney allografting surgery:	
for the first time	103 (78 %)
repeated	28 (22 %)

The prospective part included first 50 renal transplant recipients which underwent deceased donor transplantation in year 2007 and were analyzed prospectively along with diagnostics of virus infections (Table 2).

Table 2. Details of the patients in the prospective group

Patients	Index
Number of patients (n)	50
Age (years)	46.2 ± 13.8 (22 – 72)
Gender (males/ females)	26/24 (52 % / 48 %)
Body mass index (kg/m²)	24.9 ± 5.01
Basic diagnosis:	
Chronic glomerulonephritis	18 (36 %)
Chronic interstitial nephritis	13 (26 %)
Diabetic nephropathy	6 (12 %)
Polycistic kidney disease	7 (14 %)
Hypertensive nephropathy	4 (8 %)
Lupus nephritis	1 (2 %)
Unknown origin chronic kidney disease	1 (2 %)
Kidney allografting surgery:	
for the first time	41 (82 %)
repeated	9 (18 %)

During the prospective part of the study the patients were examined, treated and the diagnostics of virus infections was performed before kidney transplantation, two weeks after surgery and further every three months during the first post-transplant year, i.e., on the 3rd, 6th, 9th and 12th post-transplant month. Additionally there was analysis of patient survival and graft function made two years after transplantation.

The kidney transplantations in 50 patients of the prospective group were made from 30 deceased donors. There were both kidneys used from 20 donors and only one kidney from 10 donors. There were blood specimens from 27 donors obtained within the study, and only these donor details were analyzed (Table 3).

Table 3. Details of kidney allograft donors

Donors	Index
Number of donors (n)	27
Age (years)	45 ± 13 (19-65)
Gender (males/ females)	17/10 (63%/37%)
Body mass index (kg/m^2)	25.6 ± 3.6 (21-38)
Cause of death:	
Head trauma	8
Non-traumatic intracerebral hemorrhage	19

Patient examination methods

The patient examination included anamnesis, physical examination, determination of hematological or biochemical values for blood samples, urine analysis and graft ultrasonic examination. Ureteric stents (*Integral Ureter Stent, Rüsch*) placed in all patients were removed at two weeks. The recipients analyzed in the prospective part were additionally examined for viruses before kidney allografting surgery, as well as two weeks and 3, 6, 9 and 12 months after that. The antegrade pyelography was performed for patients diagnosed for hydronephrosis after the nephrostomy tube had been inserted in the graft. The graft biopsy was obtained from patients with suspected acute rejection or BKVN. The blood specimens of the donors described in the prospective part for virology examinations were obtained before kidney transplantation.

The graft function was assessed according to the creatinine level in the serum or according to glomerular filtration rate (GFR). The GFR was calculated according to Cocroft-Gault formula:

$$\frac{(140 - \text{age}) \times \text{body mass (kg)}}{0.81 \times \text{serum creatinine } (\mu\text{mol/l})}$$

If the patient is a female, the result must be multiplied by 0.85.

Diagnostics of hydronephrosis in the graft was performed by applying the ultrasound examination method and using LOGIQ P5 device (*General Electric*) with a semi-circle probe at the frequency of 11MHz.

Diagnostics of graft ureteral strictures was performed by applying the antegrade pyelography method, diluting 50 ml 350 mgI/ml *Omnipaque* radiopaque with 0.9% NaCl solution to 120 ml, injecting it through the nephrostome in the graft pelvis and following its flow to the bladder on the x-ray screen.

Diagnostics of CMV IgM and IgG antibodies before the transplantation was performed in donors and recipients.

For the diagnostics of decoy cells there was performed a cytological examination of urine. After the centrifugation the urine specimens were stained by Leishman's method with Azure II Eosine. The decoy cells were considered to be epithelial cells with homogenous, amorphous, ground-glass-type intranuclear virus inclusions in the centre of the nucleus and a small thick chromatin ring on the outer side of the nucleus.

Diagnostics of viral infections

In order to perform the diagnostics of BKV, CMV, HHV-6 and HHV-7 infections, the blood from the peripheral vein was collected in a blood tube with anticoagulant (EDTA), and for the diagnostics of BKV infection there was additionally collected a middle portion of the morning urine flow in a sterile urine container.

Deoxyribonucleic acid (DNA) extraction and detection of viral sequences in DNA was used to diagnose viral infections by molecular methods.

The total DNA was extracted from cell-free plasma, peripheral blood leukocytes and urine. In order to obtain the cell-free plasma, the whole blood was centrifuged for 15 minutes at 1200 – 1400 rpm, and after that, in order to sediment the cells and cell detritus – for 15 minutes at +4^{o}C and 9000 rpm. In order to obtain the DNA of the peripheral blood leukocytes, the erythrocytes were lysed by ammonium carbonate-chlorine, the blood specimen centrifuged for 10 minutes at 1200 – 1400 rpm and the leukocyte pellets were resuspended. The urine was centrifuged for 15 minutes at 3000 rpm and the sediment diluted with 200µl of the supernatant was submitted for DNA extraction. The cells were lysed by cell lysis solution containing 80 µ Proteinase K buffer, 20 µl 20% Sodium Dodecyl Sulfate solution, 15 µl Proteinase K (10 mg/ml) and 385 µl deionized water (total volume 500 µl). For DNA purification from the cell-free plasma (200 µl) *QIAamp DNA Blood mini kit* (*Qiagen GmbH*, Germany) was used according to the instructions of the manufacturer. The amount and purity of the nucleic acids were determined spectrofotometrically. The β-globin polymerase chain reaction (PCR) was used to assure the quality of the extracted DNA specimens. By assuring the quality of the DNA extracted from the plasma, a negative β-globin PCR means that the DNA specimen contains no cell admixture which is relevant when testing the reactivation of the virus. The presence of virus DNA in the peripheral blood cells, meaning leukocytes, indicates a latent persistent viral infection.

Nested, qualitative polymerase chain reaction (nPCR) was used for detection of viral sequences in DNA isolated from peripheral blood leukocytes, plasma and urine (BKV only). The detection of HHV-6, HHV-7, CMV and BKV DNA was performed according to Secchiero et al., Berneman et al., Studahl et al and Li et al, respectively. The DNA amplification products were subjected to 1.7% Agarose gel electrophoresis and visualized by UV light after staining with ethidium bromide.

Markers of the active viral infection were viral sequences in the DNA isolated from the cell-free plasma (viremia) or urine (viruria). Marker of the latent/ persistent viral infection was viral sequences in the DNA isolated from the peripheral blood leukocytes.

Biopsy of the graft

The patient is lying on his back. There is performed the ultrasound examination to mark and control the biopsy. Under the ultrasound control there is

marked the needle insertion site before the biopsy. An antiseptic solution is used for the skin, and 5 ml 2% lidocaine solution is infiltrated in the needle insertion site. The biopsy is performed with G18 automatic biopsy needle (*Tru-Core, Angiotech*). The obtained biopsy specimen is fixed in 10% formalin. There was applied the method of light microscopy in order to examine the graft biopsy specimens with hematoxiline-eosine and PAS staining.

Immunosuppression

Patients included in this study received a quadruple immunosuppression therapy: induction immunosuppression with monoclonal or polyclonal antibodies and triple maintenance immunosuppressive therapy consisting of steroids, antiproliferative agents and calcineurin inhibitors.

Induction immunosuppression was started during kidney allografting surgery, and included one of the agents that contains anti-CD25 monoclonal antibody, i.e., basiliximab (Simulect, Novartis) 20 mg intravenously on days 1 and 4, or daclizumab (Zenapax, F.Hoffmann-La Roche) 1 mg/kg intravenously on days 1 and 15, or polyclonal antibody - antilymphocyte globulin (ATG, Fresenius Biotech) 1.5 – 3 mg/kg intravenously the first 3 - 5 post-transplant days.

The initial maintenance immunosuppression in all patients consisted of methylprednisolone (Solu-Medrol, Pfizer) given intravenously in doses of 500 mg on the day of the surgery, gradually reduced within five days, followed by doses of oral prednisone (Prednisolon, Gedeon Richter) from 0.5 mg/kg per day the dose of which was also gradually reduced so that the average dose after a month were 20 mg per day and that after six months – 5 – 10 mg per day. Calcineurin inhibitors and antiproliferative drugs were instituted on the first postoperative day. Two antifroliferative drugs were used: mycophenolate mofetil (Cell-cept, F.Hoffmann-La Roche) or acidum mycophenolium (Myfortic, Novartis), at initial average dose 2 g per day orally, or azathioprine (Imuran, GlaxoSmithKline), at initial dose 100 – 150 mg per day orally adjusted per white blood cells count. Two calcineurin inhibitors were used: cyclosporine A (Sandimmun Neoral, Novartis or Ciclosporin Sandoz, Sandoz or Equoral, IVAX Pharmaceuticals) at initial average dose 3 – 4 mg/kg per day orally adjusted per its trough level in the blood: 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 (Prograf, Astellas). Tacrolimus was initiated at 0.1 mg/kg per day oral, aiming at 12-h whole blood trough level of 5 – 10 ng/ml during the first 3 months and 4 – 8 ng/ml subsequently. Patients who developed side effects of calcineurin inhibitors received sirolimus (Rapamune, Wyeth) at initial dose 2 mg per day orally, targeting trough blood level: 5 – 10 ng/ml.

All patients, who experienced 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).

Patients diagnosed for BK viremia in two consecutive measurements and/ or developing viral cytopathic changes characteristic for BKVN were assumed to have their immunosuppression reduced. In the time period between 2007 and 2008 the currently available guidelines about immunosuppression reduction schemes which are available now were not published yet, for that reason the immunosuppression was reduced with caution. The MMF dose was reduced by 500 mg per day or MMF had been 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.

Antiviral prophylaxis

Recipients exposed to a risk of active CMV infection received valganciclovir (Valcyte, F.Hoffmann-La Roche) of 900 mg or 450 mg per day orally for 3 months depending on renal graft function and the total amount of leukocytes in the blood. It was assumed that the patient is exposed to a risk of active CMV infection in the following cases:

- There were positive CMV IgM antibodies in the donor or recipient on the day of kidney transplantation.
- There were positive CMV IgG antibodies in the donor, but negative CMV IgG antibodies in the recipient.
- There were positive CMV IgG antibodies in the donor and recipient, and the recipient was additionally given methylprednisolone to treat acute rejection or received ATG for induction immunosuppression or for treating the rejection.

Data processing and statistics

The software Microsoft Excel 2003 was used for saving the collected data, Microsoft Word 2003 – for the text and tables of the thesis, and SPSS 16.0 for Windows – for the statistical analysis of the data.

There were general descriptive statistical methods used to characterize the groups. The indexes of the groups are expressed as mean values ± standard deviation (SD) or as numeric values and percentage. The obtained data were accordingly compared to t-test (Independent Samples T-Test) or Fisher's Exact Test. In order to compare the maintenance immunosuppression doses, the two way ANOVA (General Linear Model, Univariate) test was used. The difference was considered to be significant, if p-value (p) was <0.05.

In the course of the work the odds ratio (OR) was analyzed. The confidence interval (CI) was 95%. The results were considered to be statistically significant, if p-value was <0.05.

The survival of grafts and patients was analyzed by Kaplan-Meier surveillance test. The Log Rank (Mantel-Cox) test was used to compare two groups. The data were censored at the moment the patient had reached the end of the period of study.

RESULTS

Analysis of the prospective study part data

1. Frequency of the active BKV infection

The diagnostics of the virus was performed all 12 months in 46 out of 50 patients included in the study, because two patients died within the first three months of study (one due to acute pancreatitis with consecutive colonic 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.

Overall an active BKV infection during the first post-transplant year was diagnosed in 16 out of 46 (35%) patients.

The BK viruria by cytology, i.e., decoy cells in the urine, was detected in 13 out of 46 (28%) patients.

As the result of PCR testing the BK viruria was diagnosed in 16 (35%) patients, but BK viremia – in 13 (28%) patients. BK viruria and BK viremia were not always diagnosed at the same time. In the most cases BK viruria was detected first, viruria and viremia - during the next examination and after therapy modification BK viremia disappeared first and viruria – after that. The most BK viremia and viruria cases were diagnosed six and nine months after kidney transplantation, but those were not the same patients in both time periods (Table 4).

Table 4. BK viremia and BK viruria during the first year after kidney transplantation

	2 weeks after KT	3 months after KT	6 months after KT	9 months after KT	12 months after KT
Decoy cells	–	7/46 (15 %)	8/46 (17 %)	4/46 (9 %)	4/46 (9 %)
BK viruria	3/50 (6 %)	12/46 (26 %)	15/46 (33 %)	15/46 (33 %)	13/46 (28 %)
BK viremia	3/50 (6 %)	8/46 (17 %)	9/46 (20 %)	9/46 (20 %)	5/46 (11 %)
Viremia and viruria	3/50 (6 %)	7/46 (15 %)	8/46 (17 %)	9/46 (20 %)	5/46 (11 %)

In the most cases in the patients diagnosed for BK viremia by PCR there was detected also BK viruria with the same method. However three and six months

after transplantation one patient was diagnosed for BK viremia and BK viruria appeared only on the ninth month.

BK viruria was detected more often after using PCR method than during the cytological examination of urine. However in the most cases the decoy cells were found along with BK viruria and BK viremia which were detected by using PCR. This association was significant six and nine months after transplantation and almost significant (p=0.05) 12 months after transplantation (Tables 5 and 6).

Table 5. Relation of decoy cells to BK viruria diagnosed with PCR

Time period after transplantation	Decoy cells	BK viruria	Fisher's Exact (p)	Odds Ratio; 95 % CI
3 months	in 7 patients	3/7	NS	
6 months	in 8 patients	6/8	0.01	9.6; 1.65 – 56.55
9 months	in 4 patients	4/4	0.008	1.3; 1.00 – 1.85
12 months	in 4 patients	2/4	0.05	13.0; 1.32 – 127.7

Table 6. Relation of decoy cells to BK viremia diagnosed with PCR

Time period after transplantation	Decoy cells	BK viremia	Fisher's Exact (p)	Odds Ratio; 95 % CI
3 months	in 7 patients	2/7	NS	
6 months	in 8 patients	4/8	0,03	6.6; 1.23 – 35.23
9 months	in 4 patients	3/4	0,02	18.0; 1.59 – 202.9
12 months	in 4 patients	2/4	0,05	13.0; 1.32 – 127.7

One of the clinical manifestations of the active BKV infection is ureteral stricture with a consecutive hydronephrosis in the graft. During this study hydronephrosis was diagnosed in five patients. Both BK viremia and BK viruria and later also BKVN developed in three of them.

Hydronephrosis in all patients was discovered by ultrasound and as follows neprhostomy tubes were inserted in the graft and patients underwent antegrade pyelography to precise the level of ureteral obstruction. In all cases ureteral strictures was localized to the distal third of the graft ureter which is typical for strictures caused by BKV. All patients passed reoperation and neoureterocystostomy.

Hydronephrosis in the graft was diagnosed more in patients with active BKV infection (3 out of 16 or respectively 19%) than patients not diagnosed for active BKV infection (only 2 out of 30 or respectively 7%). However the difference was not statistically significant (p=0.32). During the study BKVN developed significantly more often (p=0.04) in patients with hydronephrosis than without (3 out of 5 (60%) vs 4 out of 31 (13%) respectively).

Summary

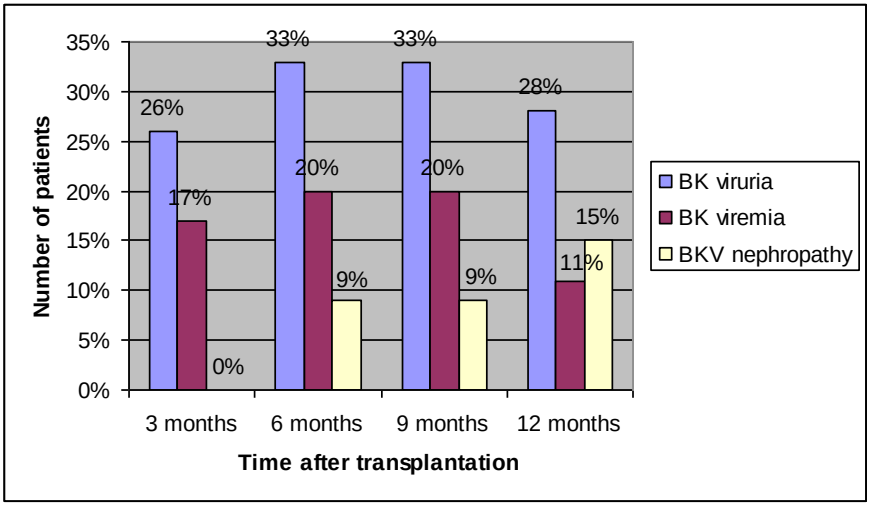
- During the first post-transplant year an active BKV infection was diagnosed in approx. one third or respectively in 16 out of 46 (35%) patients.
- An active BKV infection can rather be diagnosed by detecting BKV DNA in urine or blood specimens with PCR than by detecting decoy cells during a cytological examination of the urine.
- Ureteral strictures are a important sign of an active BKV infection and is related to a subsequent development of BKVN.

2.1. Frequency of polyoma BK virus nephropathy

BKVN was diagnosed if the patient had a persistent BK viremia, i.e., if it was diagnosed at least twice sequentially every three months and followed by graft function deterioration and/ or detection of signs typical for BKVN in the biopsy specimen.

BKVN was diagnosed in 7 out of 46 (15%) patients who were monitored 12 full months. BKVN did not develop in the first three months of the study, but six months later it was diagnosed in 4 (9%) patients having previously existing viruria and viremia. On the ninth month new BKVN cases were not diagnosed. After 12-month surveillance BKVN had developed in three (6%) more patients with a persistent BK viremia in the previous six months (Figure 1).

Figure 1. Frequency of BK viruria and viremia diagnosed by PCR and BKVN



The development of BKVN was statistically significantly related to the finding of BK viruria and viremia by PCR, but not to the finding of decoy cells 3 – 12 months after kidney transplantation (Table 7).

Table 7. Association of BK viremia, viruria and decoy cells with development of BKVN

Time period	Patients with BKVN (n = 7)	Patients without BKVN (n = 39)	Fisher's test (p)
2 weeks after KT			
BK viremia	1 (14 %)	2 (5 %)	NS
BK viruria	1 (14 %)	2 (5 %)	NS
3 months after KT			
BK viremia	5 (71 %)	3 (7.5 %)	< 0.001
BK viruria	6 (86 %)	7 (18 %)	< 0.01
Decoy cells	1 (14 %)	6 (15 %)	NS
6 months after KT			
BK viremia	5 (71 %)	4 (10 %)	< 0.01
BK viruria	7 (100 %)	9 (23 %)	< 0.001
Decoy cells	2 (28 %)	6 (15 %)	NS
9 months after KT			
BK viremia	5 (71 %)	4 (10 %)	< 0.01
BK viruria	7 (100 %)	8 (20.5 %)	< 0.001
Decoy cells	2 (28 %)	2 (5 %)	NS
12 months after KT			
BK viremia	5 (71 %)	0 (0 %)	< 0.001
BK viruria	7 (100 %)	6 (15 %)	< 0.001
Decoy cells	2 (2 %)	2 (5 %)	NS

2.2. Risk factors of Polyoma BK virus nephropathy

There were 18 risk factors defined during the study which could affect the development of BKVN:

- recipient factors: male gender, age over 50, overweight or obesity, diabetic nephropathy as the cause of end stage renal disease;
- graft factors: number of donor and recipient human leukocyte antigen (HLA) mismatches, first or repeated transplantation, cold ischemia time, primary or delayed graft function;
- immunosuppression factors: induction immunosuppression agent, acute rejection and its therapy by using methylprednisolone or ATG, tacrolimus or mycophenolate (MMF) use in the maintenance immunosuppression, usage of valganociclovir for prophylaxis of CMV infection.

The results obtained after statistical data processing are shown in table 8.

Table 8. Possible risk factors for development of BKVN

Risk factor	With BKVN (n = 7)	Without BKVN (n = 39)	(p)	Odds Ratio (CI 95%)
Gender (male/ female)	5/2	20/19	0.43	2.3; 0.41 – 13.7
Age (years)	51.7±15.9	43.6±12.9	0.11	
Patients > 50 years old	4 (57 %)	12 (31 %)	0.21	3.0; 0.58 – 15.5
BMI > 25 kg/m²	6 (86 %)	11 (28 %)	0.07	15.2; 1.64 – 141.8
BMI > 30 kg/m²	2 (28 %)	5 (13 %)	0.29	2.7; 0.41 – 18.0
Diabetic nephropathy	2 (28 %)	4 (10 %)	0.22	3.5; 0.50 – 24.32
Number of HLA mismatches	5.4±0.5	4.9±0.8	0.14	
Repeated transplantation	3 (43 %)	6 (15 %)	0.12	4.1; 0.73 – 23.29
Cold ischemia time(h)	14±4.3	13±4.8	0.64	
Delayed graft function	2 (28 %)	9 (23 %)	1.00	1.3; 0.22 – 8.07
Induction with ATG	1 (14 %)	2 (5 %)	0.39	3.1; 0.24 – 39.51
Induction with Simulect	5 (71 %)	23 (59 %)	0.4	3.3; 0.03 – 3.11
Induction with Zenapax	1 (14 %)	14 (36 %)	0.4	3.0; 0.32 – 28.8
Acute rejection	3 (43 %)	21 (53 %)	0.69	0.6; 0.12 – 3.36
ATG in rejection therapy	1 (14 %)	8 (20 %)	1.00	0.6; 0.68 – 6.15
Tacrolimus	2 (28 %)	1 (2.5 %)	0.05	15.2; 1.2 – 199.6
MMF	6 (86 %)	37 (95 %)	0.4	0.3; 0.02 – 4.15
Lack of valganciclovir	5 (71 %)	12 (31 %)	0.08	5.6; 0.95 – 33.19

A statistically significant relation to BKVN development was not detected in any of the analyzed risk factors, although some factors were observed proportionally more often in patients with BNKV.

BKVN developed more often in patients of which the maintenance immunosuppression included tacrolimus. However the statistical difference was almost insignificant (p=0.05). The tendency of a more often BKVN development was observed in patients with overweight (BMI >25 kg/m^2) (p=0.07) and patients which did not undergo CMV infection prophylaxis with valganociclovir (p=0.08). However a statistically significant difference was not discovered (Table 11).

2.3. Impact of BKVN on graft function and survival

For three patients (No. 13, 27 and 47) out of 16 diagnosed for active BKV infection the graft biopsy was not planned because there was discovered BK viruria only without BK viremia. Three patients (No. 3, 22 and 50) having a stable and good graft function were not offered to undergo graft biopsy, two patients (No.7 and 30) refused it and eight patients underwent the biopsy (Table 9).

Table 9. Graft biopsy and graft function after one year and two years in patients with active BKV infection (viruria and/ or viremia)

No.	BK viruria	BK viremia	Biopsy	BKVN in biopsy	Diagnosed BKVN	Creatinine (mmol/l) after one year	Creatinine (mmol/l) after two years
3.	present	present	not performed	-	no	0.13	0.13
7.	present	present	not performed	-	yes	0.15	0.18
8.	present	present	performed	found	yes	0.16	0.14
12.	present	present	performed	not found	no	0.13	0.12
13.	present	none	not performed	-	no	0.14	0.13
15.	present	present	performed	found	yes	0.2	HD
18.	present	present	performed	not found	no	0.09	0.1
21.	present	present	performed	found	yes	0.16	0.18
22.	present	present	not performed	-	no	0.11	0.13
24.	present	present	performed	not found	no	0.12	0.15
27.	present	none	not performed	-	no	0.13	0.13
30.	present	present	not performed	-	yes	0.14	0.17
39.	present	present	performed	found	yes	0.21	Ex.letalis
43.	present	present	performed	found	yes	0.34	HD
47.	present	none	not performed	-	no	0.12	0.11
50.	present	present	not performed	-	no	0.13	0.13

HD – hemodialysis, ex. letalis – patient died having a functioning graft, last measured creatinine level 0.24 mmol/l after 22 months.

Five out of eight patients which underwent biopsy had morphological signs typical for BKVN detected – dystrophy of epithelial cells of tubules, occasional necrosis and occasional cells with increased hyperchrome nuclei with more nucleoli.

Morphological and clinical diagnosis of BKVN were statistically significantly related ($p<0.001$; OR 3.5; 1.08 – 11.29).

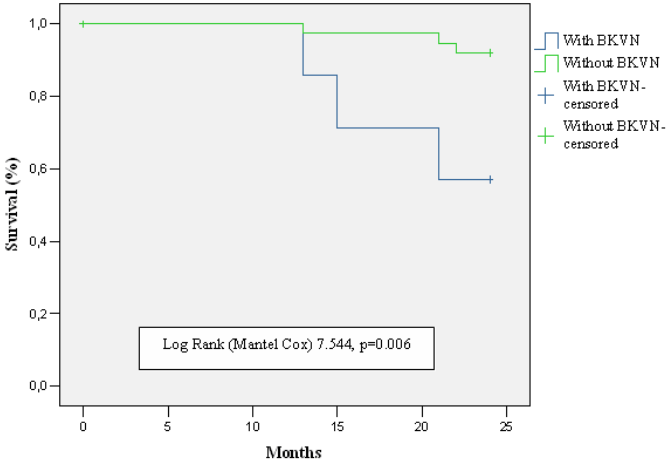
The graft function in patients with BKVN (n=7) was statistically significantly worse that the graft function in patients without BKVN (n=39) – serum creatinine level was respectively 0.19 ± 0.07 mmol/l *vs* 0.12 ± 0.04 mmol/l ($p = 0.001$) after one year and 0.16 ± 0.02 mmol/l *vs* 0.13 ± 0.03mmol/l ($p = 0.01$) after two years.

Graft survival

At the end of the first year the grafts functioned in all 46 patients which were monitored and underwent BKV infection diagnostics during the study. One year graft survival was 92% (in 46 out of 50 patients). Six grafts stopped functioning during the second year, two of them were lost due BKVN, one of them – due to acute rejection and chronic graft nephropathy and three of them – due to patient's death. In addition one of patients who died was previously diagnosed for

BKVN. Thus two years later three grafts out of seven in patients with BKVN and three grafts out of 39 in patients without BKVN (42% vs. 7%, p=0.006) did not function. Overall two-year graft survival was 80% (Figure 2).

Figure 2. Graft survival in patients with and without BKVN.



2.4. BKVN impact on hospitalization and mortality of patients

During the first year of the study participants were hospitalized 80 times including the first time of hospitalization when the kidney allografting surgery was performed. 30 times were recurrent hospital admission episodes. 26 of them were related to kidney transplantation complications and four of them – not (acute coronary syndrome, acute cholecystitis, prostate adenoma surgery and severe duodenal ulcer).

For 29 patients (58%) the first time of hospitalization was the only time they were hospitalized. During the first 12 months 15 patients (30%) were hospitalized two times, three patients (6%) – three times and three patients (6%) – four times.

Causes of a recurrent hospitalization:

- for patients with BKVN:
  - hydronephrosis – four times;
  - suspected BKVN, for biopsy – four times;
  - infection – three times (two – urinary tract infection, one – CMV infection);
  - acute rejection – two times;

- for patients without BKVN:
    - acute rejection – three times;
    - suspected BKVN, for biopsy – three times;
    - infection – two times (one – CMV infection, one – surgical wound infection);
    - lymphocele in the surgical wound – two times;
    - other causes – three times (one – hydronephrosis, one – toxic hepatitis, one - recurrence of membrano-proliferative glomerulonephritis in the graft)

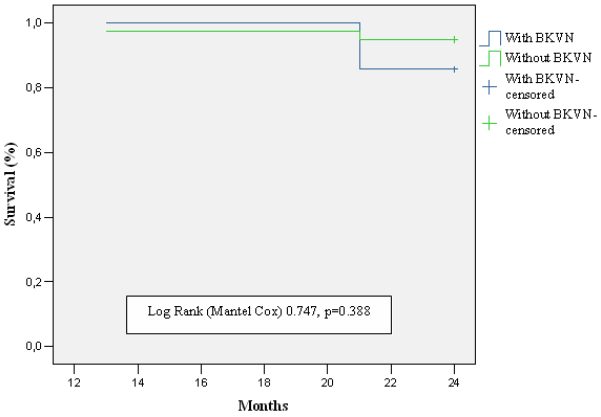
Patients with BKVN (n=7) were hospitalized statistically significantly more often than those without BKVN (n=39), respectively 3.0 ± 1.1 episode *vs* 1.4 ± 0.5 episodes within one year ($p < 0.001$). This means that the frequency of hospitalization of patients with BKVN was one case per 5 ± 3.3 surveillance months. Whereas the frequency concerning patients without BKVN was one case per 9.6 ± 3.0 surveillance months (p=0.001).

Patient survival

Two out of 50 (4%) patients died during the first three post-transplant months (one due to acute pancreatitis with consecutive colonic perforation and acute peritonitis, the other due to pulmonary artery thromboembolism). One year patient survival was 96% (48 patients out of 50). Two more patients who 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) were not included in a further monitoring and continued hemodialysis.

Thus there were 46 patients monitored during the study and all of them were alive at the end of the first study year. One year later 43 patients were alive and three – deceased. The causes of death were as follows: melanoma with multiple metastases, hepatic cirrhosis and coronary heart disease with acute myocardial infarction. The death of the patients after 24 months was statistically significantly (OR 3.0 [0.96 – 9.3], $p < 0.01$) related to a non-functioning graft after 24 months, but was not related to BKVN (p=NS). Only one of the deceased patients (cause of the death – acute myocardial infarction) was previously diagnosed for BKVN. Overall two year patient survival was 90% (45 patients out of 50) (Figure 3).

Figure 3. Patient survival in patients with and without BKVN



Summary

- During the first post-transplant year BKVN developed in 15% of the patients. Its development is not rapid. The first BKVN cases were diagnosed six months after kidney transplantation.
- Significant preconditions of BKVN are BK viremia and viruria diagnosed by PCR method.
- In the most cases a clinically diagnosed BKVN was confirmed also morphologically.
- BKVN has a significant negative impact on graft function and survival, but does not affect patient survival, although patients with BKVN are hospitalized more often thus increasing hospital costs.

3.1. Frequency of BKV and β-herpesvirus infection in renal transplant donors

Within the framework of the study there were blood specimens of 27 renal transplant donors analyzed, in order to detect latent-persistent infections or active virus infections.

An active BKV infection was not detected in the blood of donors.

Latent-persistent β-herpesvirus infection was diagnosed in 23 (85 %) donors: in seven (26 %) of them – CMV infection, in five (18.5%) – HHV-6 infection and in 19 (70 %) – HHV-7 infection. Simultaneous infection of several viruses was detected in eight (30 %) donors: three (11 %) – combination of latent-persistent CMV infection and HHV-7 infection, four (15 %) – combination of

latent-persistent HHV-6 infection and HHV-7 infection, and one (4 %) – latent infection of all three β-herpesviruses.

Active infection of β-herpesviruses was detected in four (15 %) donors: in two (7.5 %) of them – CMV infection and in two (7.5 %) – HHV-7 infection. The donors did not have clinical features of these viruses. Both recipients, to whom the kidneys were transplanted from donors with active CMV infection, received prophylaxis with valganciclovir, and primary CMV infection was developed in none of them. Both recipients, to whom the kidneys were transplanted from donors with active HHV-7 infection, had latent-persistent HHV-7 infection, and furthermore active HHV-7 infection and BKV infection was detected in one of the recipients three months after the transplantation, but in the other – six months after the transplantation.

3.2. Frequency of active BKV and β-herpesvirus infection and their interaction in renal transplant recipients

There was not only BKV infection, but also β-herpesvirus infections monitored during this study of patients after kidney transplantation.

None of the recipients was diagnosed for an active BKV infection before the kidney transplantation, but 12 (24 %) patients were diagnosed for an active β-herpesvirus infection, one (2 %) of them – for HHV-6 infection, and 11 (22 %) patients - for an active HHV-7 infection. There were no double or mixed infections detected. None of the patients had any clinical features of infections before the transplantation.

After the kidney transplantation the patients were most frequently diagnosed for an active HHV-7 infection (in cases of 26 – 37 %), quite frequently – for an active CMV infection (in cases of 2 – 26 %) and rarely – for HHV-6 infection (in cases of 0 – 13%) (Table 10).

Table 10. Frequency of active β-herpesvirus infections

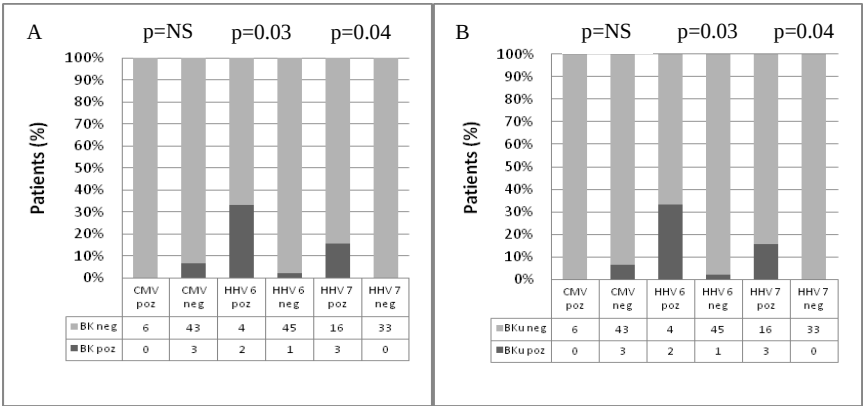
Virus infection	2 weeks after the KT	3 months after the KT	6 months after the KT	9 months after the KT	12 months after the KT
CMV	6/50 (11.5 %)	12/46 (26 %)	12/46 (26 %)	6/46 (13 %)	1/46 (2 %)
HHV-6	6/50 (11.5 %)	6/46 (13 %)	5/46 (11 %)	5/46 (11 %)	0/46 (0 %)
HHV-7	19/50(36.5 %)	14/46 (30 %)	12/46 (26 %)	17/46 (37 %)	14/46 (30 %)

During the study, 30 out of 50 (60 %) patients received valganciclovir prophylaxis for CMV infection within the first 3 months after the kidney transplantation. 19 (38 %) patients received 900 mg of Valganciclovir per day orally, but 11 (12 %) patients 450 mg of Valganciclovir per day orally due to

leukopenia or a deteriorated graft function. Within the first three months an active CMV infection developed in 7 out of 29 patients, who received valganciclovir prophylaxis, and in 7 out of 17 patients, who did not received prophylaxis (24 % vs. 41 %, p = 0.32). However six months after the transplantation new cases of active CMV infection were detected in 8 out of 29 patients, who received prophylaxis during the first three months, but no new cases of CMV infection was detected among the patient group, who did not receive prophylaxis (28 % vs. 0 %, p = 0.019). Thereby valganciclovir prophylaxis for CMV stated quite a high incidence of active CMV infection six months after the kidney transplantation.

The impact of active β-herpesviruses infections upon BKV activation was different during various study periods. Two weeks after the transplantation an active HHV-6 and HHV-6 infection was statistically significantly related to the activation of BKV during the corresponding period of time (Figure 4).

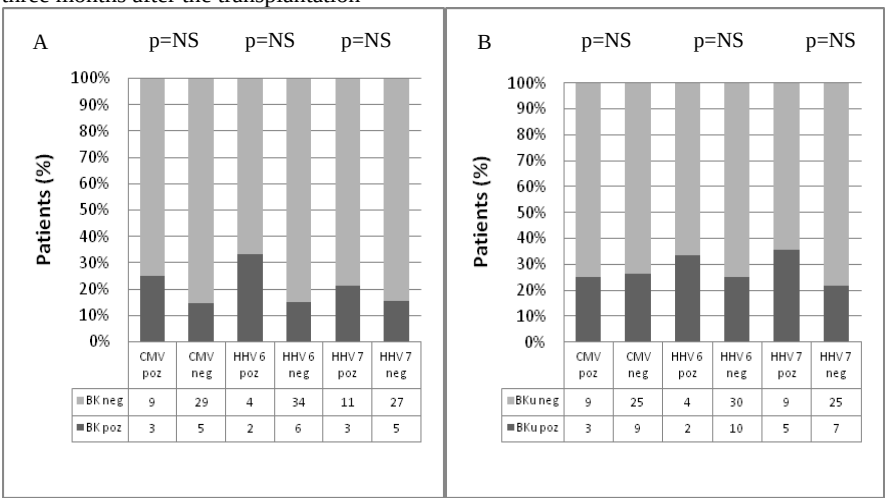
Figure 4. Relationship of active β-herpesvirus infection with the BK viremia (A) and viruria (B) two weeks after the transplantation



BK – BK viremia BKu – BK viruria

No important impact of active β-herpesviruses upon BKV activation was detected three months after the transplantation (Figure 5).

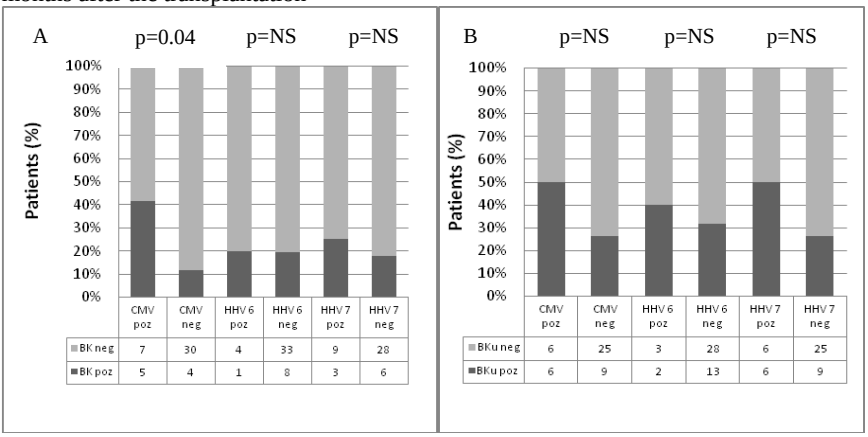
Figure 5. Impact of active β-herpesvirus infection upon BK viremia (A) and viruria (B) three months after the transplantation



BK – BK viremia BKu – BK viruria

Only active CMV infection was statistically significantly related to BK viremia (p = 0.04, OR 5.36; 1.13 – 25.26) six months after the transplantation. During this study the rest of β-herpesviruses had no impact upon BKV activation (Figure 6).

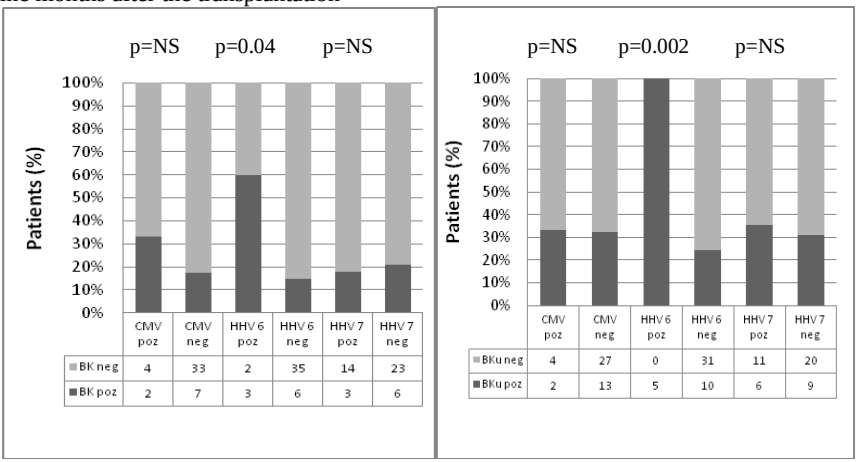
Figure 6. Impact of active β-herpesvirus infection upon BK viremia (A) and viruria (B) six months after the transplantation



BK – BK viremia BKu – BK viruria

However, active the HHV-6 infection nine months after the transplantation was related to both BK viremia and BK viruria (Figure 7).

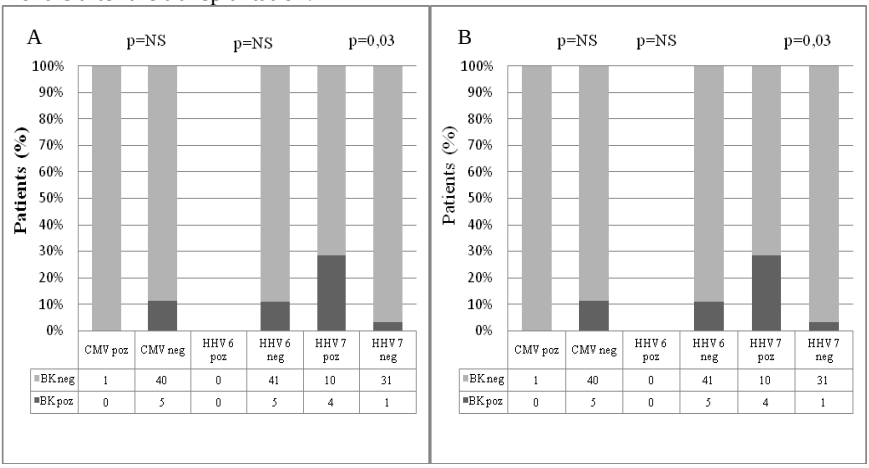
Figure 7. Impact of active β-herpesvirus infection upon BK viremia (A) and viruria (B) nine months after the transplantation



BK – BK viremia BKu – BK viruria

Only active HHV-7 infection twelve months after transplantation was statistically significantly related to the BKV activation (Figure 8).

Figure 8. Impact of active β-herpesvirus infection upon BK viremia (A) and viruria (B) 12 months after the transplantation.



BK – BK viremia BKu – BK viruria

Activation of double and mixed virus infections was first detected two weeks after kidney transplantation. An active BKV infection was diagnosed in three (6 %) recipients. Simultaneous reactivation of HHV-6 was detected in two of these recipients, furthermore, a simultaneous reactivation of HHV-7 - in all three patients. Further within the study a simultaneous activation of BKV and one of β-herpesviruses (double infection) was identified in various numbers of patients in various periods of time after the transplantation. Most frequently an active HHV-7 combined with BKV infection, rarely – active HHV-6 or CMV with active BKV (Table 14). Mixed active infection, i.e., BKV in combination with two or three β-herpesviruses infections is diagnosed more rarely than a double infection: only in one patient (2 %) three months after the transplantation, in three patients (6.5 %) after six months, and in five patients (11 %) after nine months (Figure 11).

Figure 11. Active double and mixed virus infection

Virus infections	2 weeks after the KT	3 months after the KT	6 months after the KT	9 months after the KT	12 months after the KT
CMV+BKV	0/50 (0 %)	4/46 (9 %)	6/46 (13 %)	2/46 (4 %)	0/46 (0 %)
HHV-6 +BKV	2/50 (4 %)	3/46 (6.5 %)	2/46 (4 %)	5/46 (11 %)	0/46 (0 %)
HHV-7 +BKV	3/50 (6 %)	6/46 (13 %)	6/46 (13 %)	6 (13 %)	7/46 (15 %)
Mixed	2/50 (4 %)	1/46 (2 %)	3/46 (6.5 %)	5/46 (11 %)	0/46 (0 %)

3.3. Impact of double and mixed active virus infection on graft function

During the first six months after the allografting surgery a double and mixed infection did not cause deterioration of the graft function, but after 9 and 12 months a combination of BKV and HHV-6 or HHV-7 active infection or a mixed infection, including also CMV, statistically significantly deteriorated the graft function (Table 12).

Table 12. Influence of double and mixed virus infection on kidney graft function

Virus infection	Patients with infection		Patients without infection		p
	Number of patients	Serum creatinine	Number of patients	Serum creatinine	
3 months after the KT					
CMV+BKV	4 (9 %)	0.11 ± 0.03	42 (91 %)	0.12 ± 0.03	NS
HHV6+BKV	3 (6.5 %)	0.14 ± 0.05	43 (93.5 %)	0.12 ± 0.03	NS
HHV7+BKV	6 (13 %)	0.13 ± 0.02	40 (87 %)	0.12 ± 0.03	NS
Mixed	1 (2 %)	0.09	45 (98 %)	0.12 ± 0.03	NS
6 months after the KT					
CMV+BKV	6 (13 %)	0.14 ± 0.05	40 (87 %)	0.12 ± 0.03	NS
HHV6+BKV	2 (4 %)	0.16 ± 0.09	44 (96 %)	0.12 ± 0.03	NS
HHV7+BKV	6 (13 %)	0.12 ± 0.03	40 (87 %)	0.12 ± 0.04	NS
Mixed	3 (6.5 %)	0.11 ± 0.03	43 (93.5 %)	0.12 ± 0.04	NS
9 months after the KT					
CMV+BKV	2 (4 %)	0.16 ± 0.06	44 (96 %)	0.12 ± 0.03	NS
HHV6+BKV	5 (11 %)	0.17 ± 0.03	41 (89 %)	0.12 ± 0.03	0.001
HHV7+BKV	6 (13 %)	0.15 ± 0.03	40 (87 %)	0.12 ± 0.03	0.05
Mixed	5 (11 %)	0.15 ± 0.03	41 (89 %)	0.12 ± 0.03	0.03
12 months after the KT					
HHV7+BKV	7 (15 %)	0.17 ± 0.08	39 (85 %)	0.13 ± 0.04	0.04

Grafts in patients with double virus infection lost their function proportionally more frequently, however the difference was statistically significantly only in patients with or without the combination of active HHV-6 and BKV infection (p = 0.002). Mixed active virus infection did not affect graft survival (Table 13).

Table 13. Graft survival for two years in patients with double or mixed virus infection

Combination of infections	Patients with infection		Patients without infection		*Log Rank (Mantel Cox)*
	Lost grafts	Functioning grafts	Lost grafts	Functioning grafts	
CMV+BKV	1 (17 %)	5 (83 %)	5 (12.5 %)	35 (87.5 %)	0.08, p = 0.7
HHV-6 +BKV	2 (67 %)	1 (33 %)	4 (9 %)	39 (91 %)	9.39, p= 0.002
HHV-7 +BKV	2 (18 %)	9 (82 %)	4 (11 %)	31 (89 %)	0.6, p = 0.4
Mixed	1 (11 %)	8 (89 %)	5 (13 %)	32 (87 %)	0.04, p = 0.8

Survival of patients was not affected neither by double, nor mixed active virus infection.

Summary

- BK viremia is not observed in the donors. An active CMV infection detected in the donor does not cause any complications for the recipient, if a prophylactic therapy is assigned.
- After the kidney transplantation the patients are most frequently diagnosed for an active HHV-7 infection, quite frequently – for an active CMV infection, but quite rarely – for an active HHV-6 infection.
- Reactivation of CMV occurs later in those patients, who received valganciclovir prophylaxis than in those patients, who did not receive the prophylaxis.
- A combination of an active β-herpeviruses and BKV deteriorate the graft function on the second half of the year after the surgery and cause graft loss, but it does not affect the survival of patients.

4.1. Impact of immunosuppressive therapy on the BKV activation

Patients of this study received quadruplicated immunosuppressive therapy: induction immunosuppression with monoclonal or polyclonal antibodies and triple maintenance immunosuppression that consisted of glucocorticoides, antiproliferative medication and calcineurin inhibitors.

During the observation period of 12 months, active BKV infection was diagnosed in 16 recipients, in 13 of them both BK viremia and BK viruria was detected, but in 3 recipients - BK viruria only. The number of the recipients with active BKV infection in various stages of the observance differed, wherewith also the number of patients to whom the immunosuppressive therapy was adjusted differs. Therefore its impact upon BKV activation was analyzed separately in accordance with the time periods of observance.

BKV activation (both BK viremia, and BK viruria) was detected in three patients on the second post-transplant week. Doses of immunosuppressive medication for these patients were slightly lower than for patients without BKV activation, furthermore their significant influence upon BKV activation was not observed. Average doses of immunosuppressive agents for the patients with or without BKV activation were as follows:

- dose of prednisolone: 21.6 ± 5.7 mg vs. 20.6 ± 3.7 mg per day, p = NS,
- dose of MMFl: 2000 ± 0 mg vs. 1902 ± 253 mg per day, p = NS,
- dose of cyclosporine A: 333 ± 76 mg vs. 247 ± 75 mg per day, p = NS, wherewith the level of cyclosporine A in the blood was 184 ± 13 vs. 152 ± 61 ng/ml, p = NS.

Three months after the transplantation BK viremia was diagnosed in eight patients, they have received significantly higher doses of cyclosporine A during

this period of time. Also the average trough level of cyclosporine A was higher in these patients, although the difference was not statistically significant. Three months after the transplantation BK viruria was detected in 12 patients and also they have received significantly higher doses of cyclosporine A.

Six months after the transplantation BK viremia was detected in nine patients, and it was statistically significant in relation with the higher doses of cyclosporine A within this period of time. During this period of time BK viruria was diagnosed in 15 recipients, they have received statistically significant higher doses of two immunosuppressive agents – MMF and cyclosporine A.

Nine months after the transplantation BK viremia was detected in nine patients, but it was not significantly related to immunosuppression received during this period of time. During this period of time BK viruria was detected in 15 patients and it was observed that they have received almost statistically significantly higher doses of mycofenolate mofetil and cyclosporine A.

Twelve months after transplantation BK viremia was detected in five patients, and during this period of time they had statistically significantly higher doses of prednisolone and cyclosporine A. During this period of time BK viruria remained in 13 patients, only the doses of cyclosporine A were statistically significantly higher for them than for patients without BK viruria (Table 14).

Table 14. Mean doses of immunosuppressive agents for patients with or without BK viremia and viruria 3 – 12 months after transplantation.

Time	Agent	Viremia			Viruria		
		present	missing	p-value	present	missing	p-value
3 months	Prednisone (mg)	9,6 ±1,6	9,8 ±1,3	NS	9,7 ±1,3	9,8 ±1,3	NS
	MMF (mg)	1812±258	1720 ±386	NS	1875±226	1686±396	NS
	CsA dose (mg)	275 ±82	208 ±53	0,01	275 ±63	200 ±50	<0,001
	CsA level (ng/ml)	157 ±14	140 ±32	NS	153 ±14	139 ±34	NS
6 months	Prednisone (mg)	6,9 ±2,4	6,7 ±2,1	NS	6,8 ±2,2	6,7 ±2,1	NS
	MMF (mg)	1857±243	1653 ±435	NS	1923±187	1581±446	0,01
	CsA dose (mg)	293 ±86	200 ±46	<0,001	265 ±77	196 ±49	0,001
	CsA level (ng/ml)	145 ±53	128 ±33	NS	139 ±49	128 ±31	NS
9 months	Prednisone (mg)	5,5 ±3,0	5,1 ±2,3	NS	5,7 ±3,0	4,9 ±2,1	NS
	MMF (mg)	1777±263	1634 ±436	NS	1846±240	1584±442	0,05
	CsA dose (mg)	242 ±83	204 ±55	NS	250 ±79	194 ±43	0,07
	CsA level (ng/ml)	114 ±40	129 ±38	NS	114 ±40	129 ±38	NS
12 months	Prednisone (mg)	6,5 ±2,8	3,5 ±2,6	0,02	4,4 ±2,9	3,5 ±2,6	NS
	MMF (mg)	1750±288	1609 ±438	NS	1791±257	1555±462	NS
	CsA dose (mg)	316 ±57	191 ±53	<0,001	236 ±80	187 ±49	0,02
	CsA level (ng/ml)	102 ±25	120 ±30	NS	109 ±33	9,8 ±1,3	NS

4.2. Impact of immunosuppressive therapy on the development of BKVN

The BKVN was diagnosed in 7 out of 46 (15 %) patients, who were observed for 12 months. A significant difference of the frequency of BKVN development in those patients who used various induction immunosuppression agents was not observed. Three out of 46 (6.5%) patients received polyclonal antibodies (*ATG-Fresenius*) for induction immunosuppression, and BKVN developed in one of these patients, but in two patients it did not develop (1 out of 7 (14 %) vs. 2 out of 39 (5 %), p = 0.39). 43 patients received monoclonal antibodies for induction immunosuppression: 28 patients received *Basiliximab* (*Simulect*), 15 patients - *Daclizumab* (*Zenapax*). The BKVN was detected in 5 out of 28 (18 %) patients in the group of *Basiliximab*, and in 1 out of 15 (6 %) patients in the group of *Daclizumab* (p = 0.403).

In the maintenance immunosuppression the patients received prednisone in combination with one of antiproliferative medications and with one of calcineurin inhibitors.

The dose of prednisone did not affected BKVN development in the first half-year after the kidney transplantation, but patients in who BKVN developed, had received statistically significantly higher doses of prednisone in the second half-year (Table 15).

Table 15. Doses of prednisone for patients with or without the BKVN

		2 weeks after KT	3 months after KT	6 months after KT	9 months after KT	12 months after KT
With the BKVN	Number of patients (n)	7	7	7	7	5
	Dose	22.14 ± 2.7	9.64 ± 0.9	7.5 ± 2.5	7.14 ± 3.0	5.7 ± 2.7
Without the BKVN	Number of patients (n)	39	39	39	38	30
	Dose	20.58 ± 4.2	9.87 ± 1.4	6.64 ± 2.0	4.84 ± 2.2	3.48 ± 5.7
T-test (p)		0.3	0.7	0.3	0.02	0.05

Patients both with and without the BKVN received the following antiproliferative medication in the maintenance immunosuppression – MMF or azathioprine. First month after the kidney transplantation all patients received MMF and most (86%) of the patients received a dose of 2 g per day. Three months later the usage of MMF was cancelled in one of the patients due to leucopenia, no activation of BKV or β-herpesviruses was observed in this patient during the further study. After a half of year 4 patients no longer received MMF, and MMF for two of them (one in the group of BKVN, second without BKVN) was replaced with azathioprine - 100 mg per day. Two patients with the BKVN received MMF in a reduced dose – 1500 mg per day. Nine months later the dose of MMF was

reduced to 1500 mg per day for another one patient with the BKVN. At the end of first year 4 out of 7 patients with the BKVN received MMF 1500 mg per day, two – 2000 mg per day, and one – azathioprine 100 mg per day. During the year, doses of MMF were reduced also for the part of patients in who the BKVN did not develop. It was done due to leucopenia or gastrointestinal side effects. However there was a tendency that the patients in who the BKVN develop received higher doses of MMF, than those in who the BKVN did not develop (Table 16).

Table 16. Doses of mycofenolate mofetil for patients with or without the BKVN

		2 weeks after KT	3 months after KT	6 months after KT	9 months after KT	12 months after KT
With the BKVN	Number of patients (n)	7	7	6	6	6
	Dose	1928 ± 188	1857 ± 242	1833 ± 258	1750 ± 418	1666 ± 408
Without the BKVN	Number of patients (n)	39	38	36	36	36
	Dose	1890 ± 272	1714 ± 382	1662 ± 433	1650 ± 426	1615 ± 449
T-test (p)		0.7	0.3	0.3	0.6	0.7

Patients in maintenance immunosuppression received the following calcineurin inhibitors – cyclosporine A or tacrolimus. During the first month most (96%) of the patients received cyclosporine A, but two patients already two weeks after the transplantation were treated with tacrolimus, because they were diagnosed for an acute grade III rejection previously. Three months later three patients received tacrolimus, and BKVN did not develop in two of them during the study. Tacrolimus was replaced with cyclosporine A for one patient in who the BKVN developed six months after the transplantation, but a recurrent acute grade II rejection developed and the treatment with tacrolimus was resumed. The average dose of tacrolimus for patients with BKVN was 3.6 ± 0.5 mg per day, but to the patient without BKVN – 4 mg per day (p = NS). At the beginning of the study cyclosporine A was received by 44 patients, but at the end – by 40 patients. Within the year significantly higher doses of cyclosporine A were received by patients in who a BKVN developed during the study, than by those in who it did not develop (Table 17).

Table 17. Doses of cyclosporine A for patients with or without the BKVN

		2 weeks after KT	3 months after KT	6 months after KT	9 months after KT	12 months after KT
With the BKVN	Number of patients (n)	6	5	6	5	5
	Dose	308 ± 66	310 ± 41	300 ± 44	300 ± 70	270 ± 83
Without the BKVN	Number of patients (n)	38	36	35	35	35
	Dose	257 ± 56	205 ± 53	201 ± 52	198 ± 49	191 ± 53
T-test (p)		0.05	<0.001	<0.001	<0.001	0.007

The level of cyclosporine A in the blood of patients is constantly monitored due to variations in its bioavailability. During this study the level of cyclosporine A in the blood corresponded with maintenance immunosuppression scheme accepted by Transplantation Centre of Latvia. Already three months after the transplantation there was observed the tendency that the level of cyclosporine A of patients in who later the BKVN developed was higher that of those patients in who the BKVN did not develop. Starting from the sixth post-transplantation month the level of cyclosporine A in the blood of patients with diagnosed BKVN was reduced. The level of cyclosporine A in the blood 9 and 12 months after the transplantation was lower in patients with BKVN already as a consequence of intervention, although the difference was not statistically significant (Table 18).

Table 18. The level of cyclosporine A in the blood of patients with or without the BKVN

		2 weeks after KT	3 months after KT	6 months after KT	9 months after KT	12 months after KT
With the BKVN	Number of patients (n)	6	5	6	5	5
	Level	188 ± 29	161 ± 13	145 ± 61	103 ± 36	100 ± 18
Without the BKVN	Number of patients (n)	38	36	35	35	35
	Level	159 ± 55	140 ± 32	129 ± 32	129 ± 38	121 ± 31
T-test (p)		0.2	0.1	0.3	0.1	0.1

The BKVN did not develop in two patients, who during this study received sirolimus as third component of maintenance immunosuppression.

From the fourth post-transplantation week on one patient received double (prednisone and MMF) maintenance immunosuppression and the BKVN did not develop also in this patient.

Summary

- A higher dose of cyclosporine A stated BKV reactivation during all periods of time after transplantation, and more frequent BKV activation in

the second half-year was related also to higher doses of MMF and prednisone.

- Used induction immunosuppression did not affect the development of BKVN, furthermore BKVN most frequently developed in patients who received a higher absolute dose of cyclosporine A during all first post-transplantation year with respect to its level in the blood, and a higher dose of prednisone in the second half-year after the transplantation.

Analysis of the retrospective study part data

5.1. Results of kidney transplantation for patients in the retrospective group

There was 131 adult renal transplant recipient in the retrospective group included. Similarly like surveillance of patients from the prospective group, also the surveillance of patients from the retrospective group was continued for two years in order to acquire comparative results.

During the first year 16 (12 %) kidney grafts in the retrospective group were lost. Seven of them (5 %) were lost during the first month after the transplantation (one – due to renal vein thrombosis, three – due to grade III acute vascular rejection, and three – due to primary non-functioning). Another four grafts were lost due to the death of patient (three – due to cardiovascular complications, one – due to acute hyperkalaemia), three – due to insufficient patients compliance (did not use the prescribed immunosuppressive agents), one – due to surgical complications, and one – due to sepsis. Wherewith, the one-year graft survival was 88 %.

During the second year another 16 grafts were lost. The causes of the loss of grafts were as follows: for seven – death of patients (two of them were cases of melanoma metastasis), for three – severe late acute rejection with rapidly developed chronic allograft nephropathy, for two – rapidly developed chronic allograft nephropathy only, for two – insufficient patient's compliance, and for two – infectious complications (one case of pulmonary tuberculosis recurrence, the second – recurrent graft pyelonephritis). Wherewith 32 grafts were lost within two years and the two-year graft survival was 76%. Almost a half (7 out of 16 (43 %)) of grafts lost within the second year were those of patients with repeated kidney transplantation ($p < 0.01$), but the applied induction immunosuppression and the delayed graft function did not affect the graft survival.

Grafts that were not lost had quite good function: one year later the mean serum creatinine was 0.15 ± 0.07 mmol/l or accordingly GFR 57.3 ± 20.9 ml/min, and two years later the mean serum creatinine was 0.16 ± 0.1 mmol/l or accordingly GFR 52.4 ± 25.1 ml/min. Within a year hydronephrosis was diagnosed

in 14 (12 %) patients, but chronic allograft nephropathy developed in 57 (49 %) patients.

Survival of patients in the retrospective group was comparatively good: as previously mentioned, four patients died within the first year and herof the one-year survival of patients was 96%. Another seven patients died within the second year, wherewith the two-year survival of patients was 91 %.

5.2. Comparison of patients from prospective and retrospective group

Data of patients included in the prospective and retrospective part of the study were similar (Table 18).

Table 18. Data of patients from prospective and retrospective group

	Prospective group (n = 50)	Retrospective group (n = 131)	*Fisher's Exact* (p)
Age (years)	46.2 ± 13.8 (22 – 72)	46.4 ± 12.1 (18 – 75)	NS
Gender (males/ females)	26 / 24 (52 % / 48 %)	71 / 60 (54 % / 45 %)	NS
BMI (kg/m²)	24.9 ± 5.01	24.6 ± 4.5	NS
Basic diagnosis:			
Chronic glomerulonephritis	18 (36 %)	56 (40.9 %)	NS
Chronic interstitial nephritis	13 (26 %)	20 (15.2 %)	NS
Diabetic nephropathy	6 (12 %)	18 (13.7 %)	NS
Polycystic kidney disease	7 (14 %)	21 (16 %)	NS
Hypertensive nephropathy	4 (8 %)	8 (6.1 %)	NS
Lupus nephritis	1 (2 %)	4 (3.1 %)	NS
Unknown origin chronic kidney disease	1 (2 %)	6 (4.6 %)	NS
Repeated transplantation	9 (18 %)	28 (21.4 %)	NS

The therapy assigned for patients of both study parts was similar. In accordance with the immunosuppression scheme existent in the Transplantation Centre, 19 patients from the retrospective group did not receive induction immunosuppression within the year 2005 – 2006, but starting from year 2007 the induction immunosuppression was assigned for absolutely all recipients, therefore patients from the prospective group received induction immunosuppression with *Zenapax* proportionally more frequently (Table 19).

Table 19. Immunosuppression therapy for patients from prospective and retrospective group

	Prospective group (n = 50)	Retrospective group (n = 131)	*Fisher's Exact* (p)
Induction with *Simulect*	30 (57.7 %)	69 (52.7 %)	NS
Induction with *Zenapax*	17 (32.7 %)	24 (18.3 %)	0.03
Induction with ATG	3 (6 %)	19 (14.5 %)	NS
No induction	0	19 (14.5 %)	< 0.01
Cyclosporine	40 (80 %)*	115 (87 %)*	NS
Tacrolimus	3 (4 %)*	4 (3 %)*	NS
Mycofenolate mofetil	42 (84 %)*	111 (84 %)*	NS
Azathioprine	2 (4 %)*	3 (2.3 %)*	NS
Sirolimus	2 (4 %)*	8 (6.1 %)*	NS

*Number of patients that received the corresponding medication at the end of first year

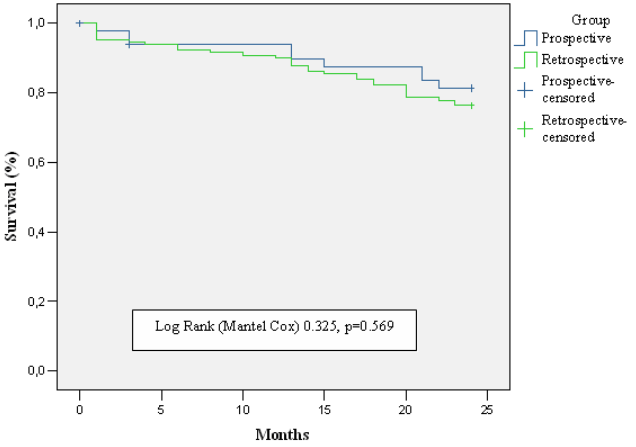
Although the induction immunosuppression differed in both study groups, the number of delayed graft function cases in both groups was equal, and the frequency of the acute rejection in the first year after the transplantation did not differ. At the end of the first year chronic changes in the graft developed in a greater number of patients from retrospective group, but the difference was insignificant. Graft function of patients from the retrospective group was also slightly, but not statistically significantly, deteriorated (Table 20).

Table 20. Clinical results of transplantation for patients from prospective and retrospective group

	Prospective group (n = 50)	Retrospective group (n = 131)	*Fisher's Exact* (p)
Delayed graft function	11 (22 %)	22 (16.8 %)	NS
Acute rejection within a year	24 (48 %)	68 (52 %)	NS
Steroid-resistant rejection	9 (18 %)	16 (12.2 %)	NS
Hydronephrosis	5 (10 %)	14 (12 %)	NS
Chronic allograft nephropathy	19 (38 %)	57 (43 %)	NS
Serum creatinine (mmol/l) one year later	0.13 ± 0.05	0.15 ± 0.07	NS
GFR (ml/min) one year later	63.6 ± 20.5	57.3 ± 20.9	NS
Serum creatinine (mmol/l) two years later	0.13 ± 0.03	0.16 ± 0.1	NS
GFR (ml/min) two years later	63.0 ± 17.3	52.4 ± 25.1	NS

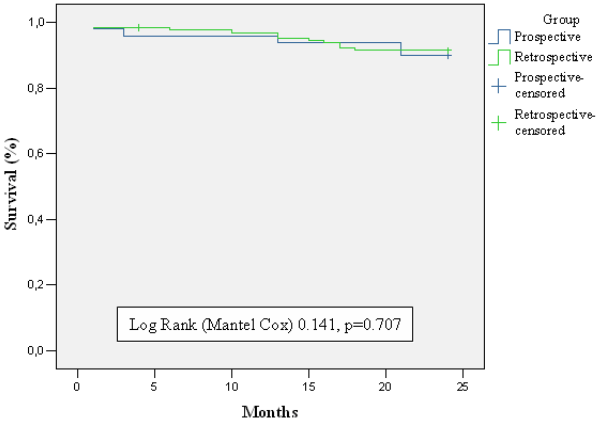
One-year graft survival in the prospective and retrospective group was respectively 92 % and 88 %, but the two-year survival – respectively 80 % and 76 %, (p = NS) (Figure 9).

Figure 9. Graft survival in prospective and retrospective study groups



In both groups the one-year survival of patients was 96 %, but the two-year survival – 90 % in the prospective and 91 % in retrospective study group (p = NS) (Figure 10).

Figure 10. Survival of patients in the prospective and retrospective study group



Summary

- The demographic data and clinical results of the transplantation were not significantly different between the patients from the prospective and retrospective group, although the induction immunosuppression in the prospective group was received by more patients.

- The graft function and survival in the retrospective group was worse than that in the prospective group, although not statistically significantly, and the survival of patients in both groups was equal.
- A regular monitoring of active BKV and β-herpesviruses infection helps to choose a more appropriate maintenance immunosuppression and improves renal graft function and survival.

DISCUSSION

Many investigators have become interested in BK virus reactivation in kidney transplant recipients, because if this infection is not diagnosed and treated timely, the BKVN develops and is followed by renal graft dysfunction and graft loss. Furthermore active BKV infection in most cases develops during the first post-transplant year and if it progress the graft is lost within the next years. During the last decade investigators have showed that it is possible to prevent the development of BKVN and the graft loss, if the active BKV infection is diagnosed and is treated early.

By applying PCR method during this study an active BKV infection was diagnosed in 16 out of 46 (35 %) patients in who the infection was specially and regularly searched during the first post-translation year. The BK viruria is the first indication of an active virus replication. By performing a cytological examination viruria was diagnosed in 13 out of 46 (28 %) patients, but with the PCR method – in all 16 patients. The tendency that less viruria cases are diagnosed with cytological methods than with molecular methods complies with data of other authors, because the determination of virus genome DNA in the urine is a more sensitive method than the diagnostics of decoy cells. In the other studies it was reported that decoy cells in the urine can be found in 12 – 16 % of renal transplant recipients, but occurrence of viruria by the PCR testing is 30 -35 % on average. It is possible that the decoy cells were diagnosed slightly more often in this study, because they were specially searched, and the answer was interpreted as positive, if only a couple of decoy cells were found, however in the papers of other authors it was considered as a positive founding if at least 10 decoy cells were found in the preparation. When active BKV infection continues, viruria is supplemented with viremia. By using the PCR method viremia was diagnosed in 13 out of 46 (28 %) patients during the year. Similar occurrence of BK viremia – 10 – 30 % - was diagnosed also by other investigators. It is considered that viremia is diagnosed in 50 % of patients within the first three post-transplantation months in who the viremia could develop within the first year. Already on the third post-transplantation month viremia was diagnosed in 8 out of 13 patients (61 %), and it indicates the same process of BKV infection development as in other transplantation centres.

At the end of year 2006, when the prospective part of this study was planned, there was an open question regarding transmission of BKV infection from the donor to the recipient similarly like in the case of CMV infection. Previously published study of Bohl et al. shows that antibodies against polyoma BK virus were diagnosed in 67 % of kidney donors by using ELISA method. Polyoma BK virus developed in 46 % of recipients who received the graft from a BK seropositive donor, and in 15 % of recipients who received the graft from a BK seronegative donor ($p = 0.007$). Furthermore BKV infection developed in 6 out of 20 recipient couples, where both recipients received grafts from the same donor, as well as sequences of the same virus genome were found in the urine and blood. This fact indicated that the BKV infection possibly originated from the donor. Therefore it was decided to test plasma specimens of donors by searching genome sequences of BKV DNA also in this study. However BK viremia was not detected in any of 27 donors examined within the study. It confirms the hypothesis supported by most of the BKV experts that BKV latently exists in the renal epithelial cells and reactivates only in circumstances of potent immunosuppression. Within this study 3 recipient couples, who had received grafts from the same donor, were observed, and at the same time active BKV infection was diagnosed. However, BKVN developed only in one recipient of each couple: BK viruria did not progress in BK viremia in one patient in the first couple, for one patient in the second couple BK viremia existed less than three months and the graft had good function, one patient in the third couple died in the third post-transplantation month due to pulmonary thrombembolism. This observation confirms that the development of an active BKV infection and BKVN is not stated only by the donor factors.

The risk factors of BK viruria, viremia and BKVN development discovered by now are very various. A too intensive immunosuppression is mentioned as the main risk factor. No statistically significant risk factor was found during this study. BKVN most frequently developed in those patients with tacrolimus in the maintenance immunosuppression scheme ($p = 0.05$, OR 15.2), to overweight patients ($p = 0.07$, OR 15.2), and to those who did not receive valganciclovir prophylaxis for CMV infection ($p = 0.08$, OR 5.6). Usage of a separate immunosuppression agent (tacrolimus or MMF) or their inclusion in immunosuppression schemes is mentioned in papers of other authors as a BKVN risk factor, but in this study the increased BMI could indicate a more intensive immunosuppression, because thus higher doses of calcineurin inhibitor are assigned. A lack of valganciclovir prophylaxis for CMV infection as a risk factor of BKVN development could be related to indirect effects of CMV infection upon the graft and the recipient, its immunomodulating effect. It is approved in the study chapter on interaction of β-herpesviruses and BKV: an active CMV infection in the sixth post-transplantation week was statistically significantly related to BK viremia, respectively, reactivation of BKV infection is provoked by changing the

immune feedback of the owner. Both β-herpesviruses, HHV-6 and HHV-7, could have similar effect upon BKV reactivation. These β-herpesviruses multiply in recipient's T lymphocytes and reduce the ability of recipient's cellular-mediated immunity to control BKV replication and promote BKV reactivation. Maybe therefore active HHV-6 and HHV-7 infection on the second week was related to the active BKV infection ($p = 0.03$ for HHV-6 infection and $p = 0.04$ for an active HHV-7 infection), active HHV-6 on the 9^{th} month with BK viremia ($p = 0.04$) and BK viruria ($p = 0.002$), and an active HHV-7 infection on 12^{th} month – with BK viremia ($p = 0.03$) and BK viruria ($p = 0.04$). During this study it was observed that combination of an active β-hrpesviruses and BKV infection within the second half of year after the transplantation significantly deteriorates the graft function and the simultaneous infection of HHV-6 and BKV contributes to graft loss ($p = 0.002$). Such data have not been observed and published previously.

Overall BKVN is diagnosed in 1 – 10 % of renal transplant recipients. Such incidence was found by other investigators when the diagnosis of BKVN was based only on morphological finding. Since a focal damage is characteristic to BKVN and negative biopsy answer does not exclude BKVN existence, there is the "presumptive" BKVN diagnosis used since 2005 as suggested by H. H. Hirsch with coauthors in the recommendations published in the journal *Transplantation*. A "presumptive" BKVN diagnosis is stated on the basis of a repeated or sustained found of viremia. Sustained finding in several studies is called viremia, which lasts for three months, but in several – for one month. During this study BKVN diagnosis was stated in 7 out of 46 (15 %) patients, who all had clinical and laboratory features that could be defined as "presumptive" BKVN, as well as features characteristic to BKV were detected also morphologically in 5 of them, i.e., 10 %. So the morphologically approved BKVN incidence within this study complies with the data of other authors. The BKVN diagnosis was stated in 4 out of 7 patients six months after the transplantation, and in 3 out of 7 patients – after 12 months, i.e., eight and a half months after the transplantation on average. Within this study the mean serum creatinine level, after diagnosing BKVN, was 0.18 (0.14 – 0.24) mmol/l. Other authors report both on higher and lower serum creatinine level. For example, in the study published by Weiss et al. on 2008, there were patients included who underwent transplantation at the beginning of the 21^{st} century, and BKVN approved in the biopsy was found in 35 out of 917 (3.8 %) patients 15 months after the transplantation on average, furthermore the average serum creatinine within the moment of diagnosing the BKVN was 2.5 mg/dl. According to Faguer et al., where BKVN was diagnosed six months after kidney transplantation on average, the mean serum creatinine level was 189 (92 – 265) μmol/l and that complies with the results of promotion work.

BKVN, expressed with graft dysfunction, can be successfully treated in 25 – 50 % of cases depending on the level of morphological damages. Furthermore graft dysfunction with higher serum creatinine can indicate an acute rejection, but

the therapy in both cases differs: in case of acute rejection the immunosuppression should be increased, but in the case of BKVN – decreased. Screening of acute BKV infection, precursor for BKV, should be performed in order to diagnose the BKVN not only in case of graft function deterioration. Recommended schemes for active BKV infection screening differs. In accordance with scheme for active BKV infection monitoring used in this study we can conclude, that examinations for detecting BK viruria and viremia once in three months time are too rare, because viruria before viremia was diagnosed only in 5 out of 13 (38 %) patients, but in 8 out of 13 (62 %) patients viremia and viruria were diagnosed at the same time. If the patients underwent examinations in order to detect viruria once a month, maybe the patients exposed to the risk of development of viremia could be identified earlier, because as it was observed in other studies, the viruria occurs approximately four weeks before viremia. By performing the screening of urine specimens it is possible to avoid from performance of useless blood tests, because viruria almost always is observed before viremia.

The optimal strategy for BKVN therapy is still an open question. However during the past years most of the investigators agree with the opinion that BKVN treatment with the reduction of maintenance immunosuppression should be started before the development of morphological changes, it means by diagnosing "presumptive" BKVN. Similarly like in case of CMV infection, such tactics of therapy is called a preemptive therapy: virus DNA appearance in the plasma is regularly monitored, and by diagnosing it, antiviral medication in case of CMV infection is prescribed, but in case of BKV the reduction of immunosuppression is started. Several authors have reported on successful usage of preemptive therapy. Preemptive therapy is also economically grounded, because money spent for the performance of virological analysis later is saved by applying reduced doses of maintenance immunosuppression agents. That was approved also in the study of Smith et al. During the promotion work the reduction of immunosuppression was performed for two patients with "presumptive" BKVN (doses of prednisone were reduced for both patients and for one – dose of MMF, and for the other – dose of tacrolimus), but the graft function of both patients continued to deteriorate (serum creatinine within the BKVN diagnosing was 0.14 and 0.15 mmol/l, after two years – 0.17 and 0.18 mmol/l). At the end of first study year after immunosuppression reduction BK viremia disappeared in 8 out of 13 (61 %) patients with diagnosed BK viremia.

Results of other studies on the impact of BKV infection on graft survival are very various. Graft survival in older studies was worse, for example, in Binett et al. study published on 1999 – 20 %, and in Randhawa et al. study – 37 %, but due to improvement of diagnostics strategy for active BKV infection and BKVN also the graft survival has improved, for example, in the study of Weiss et al. in year 2008 the one-year graft survival after diagnosing BKVN was 72,4 %, but in the study of Hardinger et al. in year 2010 the five-year survival of grafts to patients

with BK viremia – 83 %. The total one-year graft survival in the prospective group of the promotion work was 92 %, only 4 out of 50 grafts were lost, but none of them was lost due to BKVN. However the total two-year graft survival was 80 %, because the grafts with BKVN were lost during this period of time. If patients with at least once diagnosed BK viremia (n = 13) and BKVN (n = 7) are divided separately, two of them during the second year lost graft due to BKVN, but one patient with BKVN died with a functioning graft. The two-year graft survival within this study was in 10 out of 13 (77 %) patients with BK viremia and in 4 out of 7 (57 %) patients with BKVN. Graft loss in our study was greater. It could be due to a less aggressive reduction of immunosuppression, for example, the dose of mycofenolate mofetil was reduced from 2 g per day to 1.5 g, but not to 1 g per day as in other studies, or maybe due to too late started reduction of immunosuppression. The reduction of immunosuppression was commenced if the BK viremia was diagnosed at least two times with the interval of three months, or if there were cytophatic changes characteristic to BKVN found in the biopsy specimen. The fact that the reduction of immunosuppression within this study was too cautious is confirmed with similar results of data according to Ramos et al. study published in year 2002. After diagnosing BKVN the immunosuppression was reduced in 52 patients, but in 15 out of 67 patients it was not changed. Approximately after 12.6 months the graft lost was 8 out of 52 (15 %) in patients in the group, the immunosuppression of which was reduced, and in 3 out of 15 (20 %) in the group, the immunosuppression of which was not changed ($p = 0.07$). These data comply with data of our retrospective group in which the loss of grafts after one or two years was 12 % and 24 % by not monitoring active virus infections and not changing the immunosuppression.

BKV infection does not affect the survival of patients therefore only rare studies hold information about it. During the promotion work the total one-year patient survival was 96 % and two-year survival – 90 %. The two-year survival of patients with BKVN does not statistically significantly differ from patients without BKVN, it was respectively 86 % and 95 % ($p = 0.38$). According to the study of Hardinger et al. the total five-year survival of the patients was 91 %, but after comparing groups with or without BK viremia, the survival of patients with BK viremia was less – 73 % vs. 92 % ($p = 0.04$).

There are imperfections also in our study similarly like in any other study. It is mainly related to study methods that during the years 2007 and 2008 were not available in Latvia. Firstly, BK viruria and viremia was diagnosed qualitatively, not quantitatively, but also other authors do not have a joint opinion about how high viral load in the blood and urine is related to the development of BKVN. Secondly, cytophatic changes detected in biopsy specimens of the graft with light microscopy were not approved immunohistochemically. Thirdly, a reduction of immunosuppression was performed quite individually, but it should be noted, that

it was our first experience in diagnostics and therapy of complications caused by BKV infection.

CONCLUSIONS

1. During the first post-transplant year an active polyoma BK virus infection was detected in 35 % patients. BK viruria was detected more rarely using cytological examination of urine than applying the method of polymerase chain reaction.

2. Polyoma BK virus nephropathy during the first post-transplant year was diagnosed in 15 % patients. A significantly worse graft function and shorter graft survival was observed in these patients. Almost a half (42 %) of grafts with BK virus nephropathy was lost within the second year after surgery, and these patients had to return in dialysis.

3. An active polyoma BK virus infection
   - was not diagnosed in the blood of kidney donors.
   - simultaneously with active β-herpesviruses was found in 17 % cases on average, and that negatively affects the graft function.

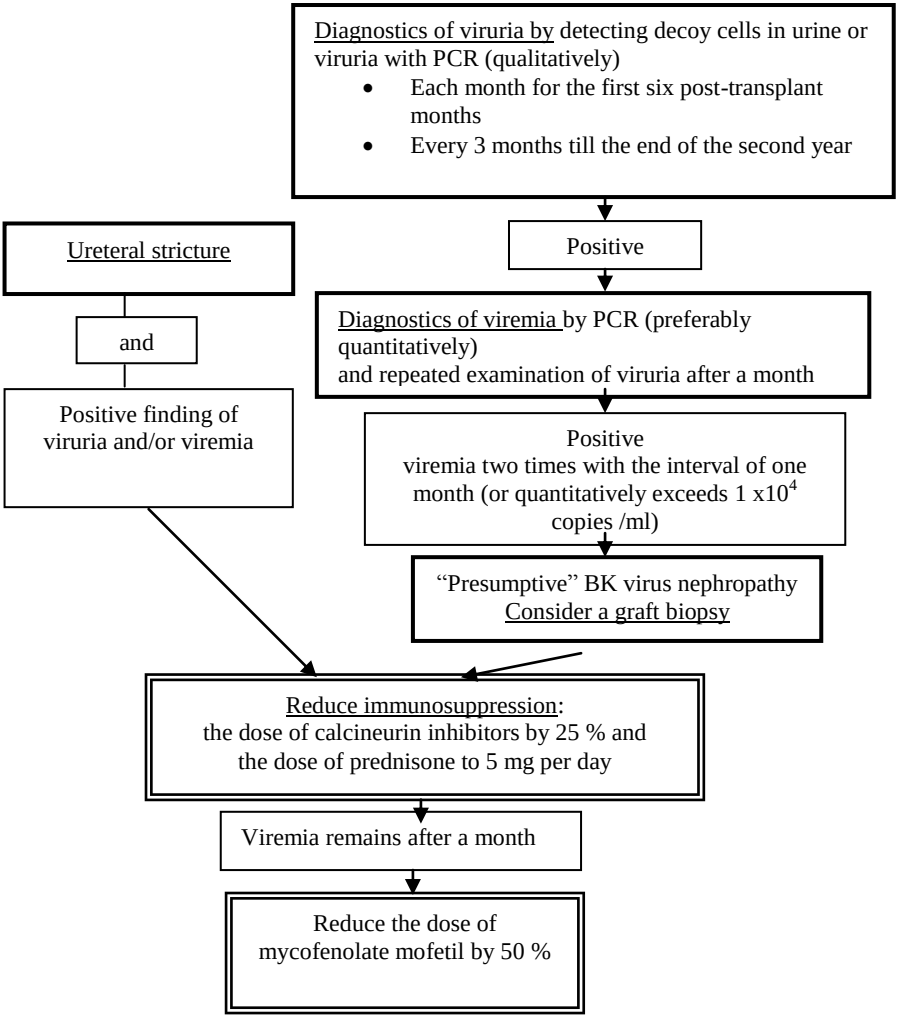
4. The activation of BK virus and the development of polyoma BK virus nephropathy were not affected by induction immunosuppression. Only a higher absolute dose of cyclosporine A from maintenance immunosuppression medications was associated with reactivation of polyoma BK virus and the development of polyoma BK virus nephropathy during the whole first year. During the second half-year after the transplantation a higher dose of mycofenolate mofetil was related to reactivation of polyoma BK virus and a higher dose of prednisone – to development of polyoma BK virus nephropathy.

5. Worse graft function and survival was observed in patients from the retrospective group in who the infections of active BK virus and β-herpesviruses were not regularly monitored. The graft function and survival is improved by performing regular diagnostics of active BK virus and β-herpesviruses infections and by choosing suitable maintenance immunosuppression, wherewith the life quality of patients improves.

PRACTICAL RECOMMENDATIONS

- Active BK virus infection should be considered as an important cause of post-transplantation complications, and the screening of it should be performed in all patients for first two post-transplant years.
- Diagnostics of BK viruria in cytological examination or detection of virus DNA in urine with polimerase chain reaction once a month for the first six months after the transplantation should be chosen as the first screening method and further it should be performed once per three months till the end of the second post-transplant year. In case of positive viruria finding, detection of viremia should be performed (preferable quantitative) and testing for viruria shoud be repeated in a month.
- The diagnosis of "presumptive" BK virus nephropathy can be stated if the viremia is positive in two measurements with the interval of one month (or quantitatively exceeds 1 x 10^4 copies/ ml). Biopsy of graft by taking two kidney tissue specimens from various places of the graft should be considered when a "presumptive" BK virus nephropathy is diagnosed, and the reduction of immunosuppression should be started in accordance with scheme in respect to fact whether the diagnosis of polyoma BK virus nephropathy is proved in the biopsy.
- Reduction of immunosuppression in case of a "presumptive" BK virus nephropathy diagnosis should be initiated by reducing the dose of calcineurin inhibitor by 25 % regardless of it level in the blood and by reducing the dose of prednisone to 5 mg per day. If the viremia does not disappear after one month, the dose of mycofenolate mofetil should be reduced by 50 %.
- In accordance with the previously mentioned scheme the reduction of immunosuppression should be initiated as well in patients with a diagnosed ureteral stricture and one positive finding of viruria or viremia by using the polymerase chain reaction testing (Figure 11).

Figure 11. Scheme of screening and therapy for active BK infection



THE LIST OF PUBLICATIONS

Original articles:

- Ziedina I, Folkmane I, Chapenko S, Murovska M, Sultanova A, Jushinskis J, Rozental R. Reactivation of BK Virus in the Early Period After Kidney Transplantation. *Transplantation Proceedings* 2009, 41: 766-768.

- Chapenko S, Folkmane I, Ziedina I, Chistyakovs M, Rozentals R, Krumina A, Murovska M. Association of HHV-6 and HHV-7 reactivation with the development of chronic allograft nephropathy. *Journal of Clinical Virology* 2009; 46(1): 29-32

- Ziediņa I., Čapenko S., Folkmane I., Murovska M., Čistjakovs M., Jušinskis J., Rozentāls R. Herpesvīrusu, parvovīrusa B19 un poliomas BK vīrusa infekcija nieres transplantātu donoriem *RSU Zinātniskie raksti* 2009: 22-27.

- Ziediņa I., Čapenko S., Folkmane I., Sultanova A., Murovska M., Jušinskis J., Rozentāls R. Poliomas-BK vīrusa un beta-herpesvīrusu reaktivācija pēc nieres transplantācijas. *RSU Zinātniskie raksti* 2008: 81-86.

- Ziediņa I., Čapenko S., Folkmane I., Sultanova A., Murovska M., Rozentāls R. Poliomas-BK vīrusa un citomegalovīrusa vienlaicīga infekcija pacientiem ar hronisku transplantāta nefropātiju. *RSU Zinātniskie raksti* 2007: 39-43.

Abstracts:

- Ziedina I, Folkmane I, Chapenko S, Murovska M, Sultanova A, Jushinskis J, Rozental R. Polyoma BK virus in kidney transplant recipients. *Final programme & abstract book of the 10th Baltic Nephrology Conference*, 2010, O, Abstract book: 2.

- Ziedina I, Chapenko S, Folkmane I, Jushinskis J, Murovska M, Rozental R. Co-infection of Polioma BK virus and β-herpesviruses in Kidney Transplant Recipients. *Abstracts of the Scandinavian Transplantation Society XXV Congress*, 2010, O-30, Abstract book: 54.

- Ziedina I, Chapenko S, Folkmane I, Jushinskis J, Suhorukov V, Murovska M, Rozental R. Association of induction immunosuppression with viral infections and kidney graft function. *Abstracts of 9th International Conference on New Trends in Immunosuppression & Immunotherapy* 2010, P-86, Abstract book: 47.

- Ziediņa I., Folkmane I., Čapenko S., Murovska M., Jušinskis J., Rozentāls R. Herpesvīrusu, parvovīrusa B19 un poliomas BK vīrusa infekcija nieres transplantātu donoriem. *RSU Zinātniskā konference* 2009, Tēzes 92. lpp.

- Folkmane I, Chapenko S, Kozireva S, Ziedina I, Rozentals R, Murovska M. Prevalence of blood-borne viral infection among renal transplant donors. *Abstracts of 5th ETCO meeting. Organs, Tissues & Cells* 2009, 12(1): 61.

- Ziedina I, Folkmane I, Chapenko S, Murovska M, Sultanova A, Jushinskis J, Rozental R. Reactivation of BK Virus in Early Period After Kidney Transplantation. *The Scandinavian Transplantation Society XXIV congress*, 2008, P-43, Abstract book: 72.

- Folkmane I, Ziedina I, Rozental R, Chapenko S, Murovska M. Impact of early beta-herpesviruses infection activation on chronic allograft nephropathy development. *Clinical Virology annual Meeting* 2008, P5-08, Abstract book: 40.

- Ziediņa I., Folkmane I., Čapenko S., Murovska M., Sultanova A., Jušinskis J., Rozentāls R.. Poliomas BK vīrusa un beta-herpesvīrusu reaktivācija agrīnā periodā pēc nieres transplantācijas. *RSU Zinātniskā konference* 2008, Thesis p.122.

- Ziedina I, Chapenko S, Folkmane I, Sultanova A, Murovska M, Rozental R. Co-infection of Poliomavirus-BK and Cytomegalovirus in Renal Transplant Recipients with Chronic allograft Nephropathy. *RSU Zinātniskā konference* 2007, Thesis p.168.