

THE PREVALENCE OF THE MOST IMPORTANT VIRAL INFECTIONS IN RENAL TRANSPLANT RECIPIENTS IN SERBIA

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Abstract - Viruses are the main cause of opportunistic infections after kidney transplantation. The aim of this study was to determine the prevalence of cytomegalovirus (CMV), Epstein-Barr virus (EBV), B. K. virus (BKV) and John Cunningham virus (JCV) infections in renal transplant recipients (RTR). This retrospective study of 112 RTR investigated the presence of CMV, EBV and polyomaviruses DNA in plasma and/or urine by PCR. The visualization of PCR products was performed by electrophoresis on 2% agarose gel stained with ethidium bromide and photographed under a UV light. The chi-square test was used for statistical analysis. CMV DNA was detected in 14/112 (12.5%), EBV DNA in 4/49 (8.16%), BKV DNA in 10/31 (32.26%) and JCV DNA in 3/31 (9.68%) RTR. These results show that CMV infection is more often present in RTR compared to other investigated viral infections. In the light of these results, molecular testing could be useful in identifying recipients at high risk of symptomatic post-transplant viral infection.

Key words: Kidney transplantation, viral infections, renal transplant recipients, viral and molecular diagnostic procedures

INTRODUCTION

The human body has a complex system of protective mechanisms to prevent infection which involves both adaptive and innate immune systems (e.g. skin, mucus membranes). Immunocompromised patients have one or more defects in their body's defense against microbial invaders. They can become infected with pathogens capable of infecting immunocompetent individuals, but also with opportunist microbes that do not cause diseases in healthy individuals, but are capable of infecting an immunocompromised person, often leading to severe and life-threatening infections with fatal consequences. Different types of defects can predispose individuals to infections with different pathogen, depending on the critical mechanisms operating in

defense against individual types of microorganisms (Fishman, 2007; Blair et al., 2008).

Solid organ and bone marrow transplantation are among the most important advances in modern medicine. Their success depends to a great degree on the ability to control and manage the adverse effects of immunosuppressive regimens, primarily the susceptibility to infection as long as immunosuppression is applied. Although renal, cardiac and hepatic allografts would appear to employ very different procedures, they result in similar patterns of transplant-related infection. The reason for this apparent paradox is that the risk of infection is largely the result of the immunosuppressive treatment given to prevent acute and chronic rejection. This treatment is in principle standardized for all solid organ transplants. The

risk of infection increases with the extent and duration time of the treatment; consequently, infections can be related to the time of organ transplantation. Immunosuppressive therapy such as cyclosporine, glucocorticoids and antilymphocyte globulin, combine to induce a broad spectrum of immune defects (Kotton and Fishman, 2005).

Despite significant advances in the field of renal transplantation, long-term graft survival is not dramatically increased (Meier-Kriesche et al., 2004). The reasons for this are various, but include the persistent impact of infectious diseases on transplant recipients. Viral infections continue to be a potential contributor to graft failure, and a cause of severe morbidity and mortality (Hartmann et al., 2006; Toyoda et al., 2005). The consequences of viral infections are variable and may include direct involvement of the allograft, dissemination to other organs or indirect effects on the patient and allograft. Some viruses, notably herpesviruses and polyomaviruses, are believed to further impair host defense, thereby increasing the risk for other infections. Viral infections have also been implicated as cofactors in acute and chronic rejection syndromes (Toyoda et al., 2005; Sageda et al., 2002).

According to the time elapsed since transplantation and the risk status of the patient, the post-transplant period is divided into three phases: an early period (phase one) usually lasting less than 4 weeks, a middle period (phase two) from the first month until the first year, and a late period that lasts more than one year from the time of transplantation. The risk of infection after transplantation depends on a variety of factors such as intensity, virulence and mechanisms of viral exposures, the nature of the immunosuppressive regimen and presence or absence of pre-existing antiviral immunity. During the first month, bacterial and nosocomial infections caused by antimicrobial resistant microbes predominate. Between one and six months post-transplant, there is a risk of viral infection. Patients with a history of herpes simplex virus (HSV) infections may have early reactivation (1 or 2 months after transplantation). Infections acquired from the donor, such as HBV, HCV or

HIV, may also appear in the first month after surgery. Cytomegalovirus (CMV), both reactivation and new disease acquired in the middle post-transplant period, tend to appear in months 1 to 4 or after the cessation of antiviral prophylaxis (approximately by day 100). Other latent viruses such as the Epstein Barr virus (EBV) and varicella zoster virus (VZV) also tend to appear in months 2 to 6. Half of BK polyomavirus nephropathy is observed in the first 6 months after transplantation while the other half occurs at a later time. Community acquired infections such as influenza and respiratory syncytial virus (RSV) can appear at any time after transplantation (Razonable et al., 2003). In recent years, the significance of adenovirus and human metapneumovirus infections is increasingly recognized (Camille et al., 2005).

Viral infections remain an important cause of morbidity and mortality in transplant recipients. They may acquire these infections from the donors (donor-derived infections), from reactivation of an endogenous latent virus, or from the general community.

The effects of viral infection are classified as either "direct" or "indirect". This classification helps in separating the effects of invasive viral infection (cellular and tissue injury) from effects mediated by inflammatory responses (e.g. cytokines) or by alterations in host immune and inflammatory responses (Rubin, 2002; Ljungman, 2002). Syndromes such as fever and neutropenia (e.g. with CMV infection) or invasive disease resulting in pneumonia, enteritis, meningitis or encephalitis are considered direct effects. Indirect effects of viral infections include responses to viral infections such as the release of cytokines, chemokines or growth factors. These effectors are immunomodulatory, resulting in further immune suppression and increased risk of other opportunistic infections (Boeckh and Nichols, 2003). In addition, viral infections alter the expression of surface antigens (e.g. histocompatibility antigens), provoking graft rejection and/or causing dysregulation of cell proliferation (contributing to oncogenesis). Infections with one virus may cause immune suppression or otherwise stimulate the replication of

other viruses in a form of viral “cross-talk” (e.g. CMV and HCV). Multiple studies implicate infection with the human herpes virus (HHV) 6 and 7 as a risk factor for CMV disease, while CMV may in turn trigger HHV6/7 reactivation (Tong et al., 2000; Kidd et al., 2000; Dockrell and Paya, 2001). Increased viral replication and persistence may contribute to allograft injury like fibrosis or chronic rejection (Babel et al., 2001).

The risk of viral infection in these patients depends on several factors, such as the type of organ transplanted, the intensity of immunosuppression, the recipient’s susceptibility and donor/recipient (D/R) serological status. Direct infection of allografts by viral pathogens is common and can cause extensive damage to the graft tissue. In some cases, some infections may contribute to other complications, such as acute or chronic rejection of the transplant organ. In addition to these direct effects, viral infections cause indirect effects, including a greater risk of the replication of other viruses, graft rejection, and opportunistic infections with specific entities for each types of transplant. These indirect effects result from the immunomodulatory activity of some viruses, such as CMV and HHV6. Solid organ and hematopoietic stem cell transplant recipients are uniquely predisposed to severe clinical manifestations, due to the variety of common and opportunistic viruses (Castagnola et al., 2008).

The maintenance of immunosuppressive therapy to prevent rejection also predisposes transplant recipients to systemic infections with a range of pathogens. Some of these, including CMV and adenovirus, can also infect the allograft, while others, such as Parvovirus B19, exhibit tropism for the bone marrow cells that typically do not involve the transplant itself. In renal transplant, infections are associated with a spectrum of glomerulonephritis, many mediated by immune complexes (Djamali et al., 2006).

Viral infections cause a wide variety of complications in solid organ transplant recipients, some of them life threatening. Herpes viruses, most notably CMV and EBV, are the most common among op-

portunistic viral pathogens that cause infection after solid organ transplantation.

CMV is the major microbial pathogen with a negative effect in solid organ transplant (SOT) recipients. The majority of donors and recipients have latent CMV infection at the time of transplantation (Zhang et al., 1995). Furthermore, CMV is a cell-associated disease, with the viruses primarily latently residing in T lymphocytes, although particles can also be found in polymorphonuclear cells, endothelial vascular tissue and renal epithelial cells. This cell specificity allows transmission of the virus within the transplant organ (Crumpacker and Wadhwa, 2005). The cytomegalovirus can substantially impact host immune responses. Following infection, CMV infiltrates the cell and produces immediate-early antigens (Ag) that regulate DNA transcription, and during the next 6 to 24 h, CMV produces late Ag which direct nucleocapsid protein production. It also causes upregulation of IL2 and downregulates MHC class I molecules on the surface of infected cells allowing them to evade host immune recognition (Brennan, 2001). When compared with other organ transplant recipients, renal transplant recipients are at lower risk of CMV infection. The incidence of CMV in the renal transplant population is estimated to be between 8 and 32%. Donor seropositivity, especially in the absence of prior recipient infection, is the most important risk factor for post-transplant infection. CMV seronegative recipients of seropositive kidneys are at increased risk of invasive CMV disease, recurrent CMV, and ganciclovir-resistant CMV infection (Liapis et al., 2003; Paya et al., 2004). The presentation of CMV infection may be variable, ranging from asymptomatic infection defined by the presence of active viral replication to end organ or disseminated organ involvement (Kotton et al., 2005; Barzon et al., 2009).

EBV is responsible for some cases of post-transplant lymphoproliferative disorder (PTLD), which represents the most serious complication in transplant recipients. It was recently recognized that proliferating cells may be of either host or donor origin, with possible prognostic implications. Renal trans-

plantation recipients have the lowest risk of acquiring PTLD in comparison with other transplant patients (approximately 1-3%). PTLD most commonly occurs in the first year post-transplant. Serology for the presence of EBV specific antibodies should be obtained before transplantation. Allograft recipients who are EBV negative before transplantation and receive an organ from a seropositive donor are at greatest risk for PTLD (Green et al., 2006); consequently, this is most commonly observed in pediatric and young adult populations. PTLD can be present in many organs, including the allograft itself (Opelz et al., 2006).

The BK polyomavirus that was first described in a renal transplant recipient with ureteral stenosis, has recently been recognized as an important cause of renal graft impairment. The prevalence of several forms of virus infection in allograft recipients has shifted considerably over the last ten years. Since the mid 90's, polyoma BKV interstitial nephritis, previously rare, has become an important cause of renal transplant dysfunction and/or loss of graft. The BK virus typically involves the allograft kidney, with manifestations including intestinal nephritis, ureteral stenosis and ureteral stricture. Most common infection occurs within the first three to four months after transplantation, when immunosuppression is at its highest (Randhawa and Brennan, 2006).

The aim of this study was to determine the prevalence of the most important viral infections in renal transplant recipients: CMV, EBV and polyoma BKV and JCV.

MATERIALS AND METHODS

Patients

Renal transplant recipients from the Transplantation Center of the Clinic of Nephrology, Clinical Center of Serbia, Faculty of Medicine, University of Belgrade, were retrospectively reviewed from January 2010 to December 2011. Samples (plasma and urine) were tested for the presence of CMV, EBV and polyoma BKV and JCV DNA in the Virology Laboratory of

the Institute of Microbiology and Immunology, Faculty of Medicine, University of Belgrade. A total of 112 kidney recipients, 63 adults (36 male, median age 45,89; range 22-63 and 27 female median age 45,44; range 23-60) and 49 children (33 boys, median age 14,4; range 3-21 years, and 16 girls median age 15,81; range 6-21) were included in this study. All patients belong to the middle post-transplant period. The donor/recipient CMV serological status was known for only 33 of the 112 transplant recipients (D/R CMV IgG+, IgM-).

All 112 patients were monitored for CMV DNA, 49 for EBV DNA in plasma samples, and 31 transplant recipients were investigated for BKV and JCV DNA in plasma samples and urine using a PCR-based test. Blood samples were collected in 5 ml Vacutainer[®] tubes containing EDTA, and after centrifugation DNA was extracted from 200 µl of plasma using the QIAamp Blood Mini Kit (QiAGEN GmbH, Hilden, Germany), while DNA from the urine samples was extracted using the QIAamp DNA Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's protocols.

Isolation and amplification of viral DNA

CMV DNA was amplified using PCR targeting of a 435 bp region in exon 4 of the Major Immediate Early (MIE) gene. The amplification was performed using the following primers: FW 5'-CCAAGCG-GCCTCTGATAACCAAGCC-3' and REV 5'-CAG-CACCATCCTCCTCTTCCTCTGG-3'. PCR amplification was run in a reacting volume of 25 µl containing 12.5 µl PCR Master Mix (QIAGEN Taq PCR Master Mix, Hilden Germany), 1 µl (1 µM) FW and 1 µl (1 µM) REV primers, 5 µl previously isolated DNA and 5.5 µl injection grade water. PCR was carried out in a thermocycler Master Cycler Gradient (Eppendorf, Germany) following several steps: an initial denaturation at 95°C for 5 min, followed by 35 cycles at 95°C for 60 s, 58°C (60 s), 72°C (90s), and terminal elongation at 72°C for 10 min.

For EBV DNA detection, two different set of primers were used. The first set amplified a 269 bp

within the EBNA 1 coding region (FW 5'-GTCAT-CATCATCCGGGTCTC-3'; REV 5'-TTCGGGTT-GGAACCTCCTTG-3'), and the second set of primers (FW 5'-GGCGGCTGGTGTACCTGTGT-TA-3'; REV 5'-CCTTAGGAGGAACAAGTCCC-3') amplified a 239 bp within the gp220 coding region of the EBV genome. The PCR program for amplification of EBNA1 and gp220 EBV DNA employed an initial denaturation step at 94°C for 10 min, 32 cycles of denaturation at 94°C for 90 s, annealing at 60°C for 45 s and extension at 72°C for 2 min, with a final extension step at 72°C for 10 min. PCR was performed using a thermocycler Master Cycler Gradient (Eppendorf, Hamburg, Germany).

The 116 bp fragment within the VP2 coding region of the BKV and JCV genome was amplified by using a common forward primer for BKV and JCV (5'-TGCTCCTCAATGGATGTTGC-3'), and different reverse primers (BKV 5'-ATTGAGGAGCAGT-TCTT-3'; JCV 5'-CACGGGGTCTTCTTTC-3'). The PCR program, for amplification of both viruses, was initial denaturation at 95°C for 5 min, 40 cycles of denaturation at 95°C for 30 s, annealing at 50°C for 40 s and extension at 72°C for 1 min, with the final extension step at 72°C for 10 min.

Visualization of PCR products of appropriate lengths was performed by electrophoresis in 2% agarose gel stained with ethidium bromide.

Statistical analyses

The chi-square test was used for statistical analyses. *P* values <0.05 were considered statistically significant and *P* values <0.01 were considered highly statistically significant.

RESULTS

CMV DNA was tested in plasma samples in all 112 kidney recipients (63 adults and 49 children) included in this study. CMV DNA was detected in 14/112 tested renal transplant patients (12.5%) (Table 1). Thirteen of 14 CMV DNA-positive patients were adults (10 male and 3 female) and one of 14 with a

CMV DNA-positive finding, was a child (Table 2). Statistical analyses between two subgroups of tested patients showed a highly statistically significant difference in the prevalence of CMV active infection between adults and pediatric patients ($\chi^2=8.322$; $P<0.01$). On the other hand, there was no statistically significant difference in the frequency of CMV-positive PCR between men and women ($\chi^2=2.472$; $P>0.05$).

Forty-nine blood samples from pediatric patients were tested for the presence of EBV DNA by PCR and only 4/49 (8.16%) samples were positive (Table 3). Three of four EBV-positive patients were younger than 15 years of age, and one transplant recipient was over fifteen years old. Statistical analyses did not show a significant difference in the frequency of EBV DNA detection in two groups of pediatric patients ($\chi^2=1.340$; $P>0.05$) (Table 4).

Even though EBV DNA was detected in four compared with one CMV DNA-positive result in the tested pediatric transplant recipients, no statistically significant difference between the frequency of CMV and EBV DNA detection was found in the pediatric population ($\chi^2=1.896$ $P>0.05$) (Table 5).

BKV and JCV DNA were detected in 13 of a total of 62 (20.9%) blood and urine samples collected from 31 renal transplant recipients. BKV DNA was detected in 10/31 (32.26%) patients while JCV DNA was detected in 3/31 (9.68%) patients. Statistical analyses showed that there was a statistically significant difference in the detection of investigated polyoma viruses in the kidney transplant recipients included in this study ($\chi^2=4.770$; $P<0.05$) (Table 6). While these results suggest that polyoma viruses viruria (8/62; 12.9%) represents a more frequent finding than viremia (5/62; 8%) in renal transplant recipients, statistically there was no significance difference ($\chi^2=2.296$; $P>0.05$; $\chi^2=1.958$; $P>0.05$ respectively) (Tables 7 and 8).

Comparison between the groups of CMV DNA-positive (14/112) and BKV DNA-positive results (10/31), and CMV DNA (14/112) and JCV DNA-

Table 1. CMV DNA finding in plasma sample of investigated renal transplant patients

	CMV positive	CMV negative	Total
Adults	13	50	63
Children	1	48	49
Total	14	98	112

$\chi^2 = 8,322$
 $p < 0.01$

Table 2. CMV viremia in examined male and female renal transplant recipients

	CMV positive	CMV negative	Total
Man	10	26	36
Women	3	24	27
Total	13	50	63

$\chi^2 = 2.472$
 $p > 0.05$

Table 3. Epstein-Barr (EBV) viremia in tested renal transplant pediatric recipients

	EBV positive	EBV negative	Total
Boys	3	30	33
Girls	1	15	16
Total	4	45	49

$\chi^2 = 0.111$
 $p > 0.05$

Table 4 Correlation of EBV viremia and age of renal transplant pediatric recipients

	EBV positive	EBV negative	Total
<15 years	3	20	23
>15 years	1	25	26
Total	4	45	49

$\chi^2 = 1.340$
 $p > 0.05$

Table 5 Intergroup comparison between CMV and EBV DNA in renal transplant pediatric recipients

	Positive	Negative	Total
EBV	4	45	49
CMV	1	47	48
Total	5	92	97

$\chi^2 = 1,896$
 $p > 0.05$

Table 6. BKV and JCV PCR findings in plasma and urine samples of tested renal transplant recipients

	Positive samples	Negative samples	Total
BKV	10	21	31
JCV	3	28	31
Total	13	49	62

$\chi^2 = 4.770$
 $p < 0.05$

Table 7. BKV and JCV PCR findings in urine samples of tested renal transplant recipients

	Positive (urine)	Negative (urine)	Total
BKV	6	25	31
JCV	2	29	31
Total	8	54	62

$\chi^2 = 2.296$
 $p > 0.05$

Table 8. BKV and JCV DNA PCR findings in plasma samples of tested renal transplant recipients

	Positive (plasma)	Negative (plasma)	Total
BKV	4	27	31
JCV	1	30	31
Total	5	57	62

$\chi^2 = 1.958$
 $p > 0.05$

Table 9. CMV and BKV findings in tested renal transplant recipients - intergroup comparison of prevalence of infections

	Positive	Negative	Total
CMV	14	98	112
BKV	10	21	31
Total	24	119	143

$\chi^2 = 6.795$
 $p < 0.05$

Table 10. CMV and JCV findings in tested renal transplant recipient - intergroup comparison of prevalence of infections

	Positive	Negative	Total
CMV	14	98	112
JCV	3	28	31
Total	17	126	143

$\chi^2 = 0.192$
 $p > 0.05$

positive patients (3/31), showed that there was a statistically significant difference between the frequency of CMV and BKV DNA detection in renal transplant recipients ($\chi^2=6.795$; $P<0.05$) (Table 9). At the same time, in the other comparison groups, CMV/JCV DNA-positive patients, there was no statistically significant difference ($\chi^2=0.196$; $P>0.05$) (Table 10).

DISCUSSION

Viral infections are a serious complication in kidney transplant recipients and represent the primary cause of hospitalization, especially among pediatric recipients who are often seronegative at the time of transplantation (Kotton and Fishman, 2005). In these patients, viral infections usually occur very soon after transplantation, and in many cases are transmitted from transplanted tissues and blood cells, where viruses exist in latency, or as a result of active infections of the donor that are unrecognized at the time of organ procurement (Fishman, 2007). The presence of viruses in the graft depends on their tropism for specific tissues and cells and their ability to establish latency or persistent infection. This is the case for viruses such as CMV, EBV and recently demonstrated polyoma BKV and JCV (Barzon et al., 2009) that can cause, besides life-threatening diseases, injury of the renal graft.

CMV remains the most important viral pathogen after transplantation despite the availability of effective antiviral drugs and validated strategies for prophylactic, preemptive and therapeutic therapy. CMV can affect almost every organ and system, with frequent recurrences and increasing rates of antiviral resistance. Together with indirect long-term effects, CMV significantly reduces graft and patient survival after solid organ transplantation. According to the results of Blair and Blumberg (2008), the prevalence of CMV infection in renal transplant recipients is estimated to be between 8% and 32%. Results from this study showed that the prevalence of CMV active infection is 12.5% (14/112 patients were CMV DNA positive). Also, findings from this study indicate a statistically significant difference in the prevalence of CMV DNA-positive results in plasma sam-

ples between adults and pediatric kidney transplant recipients ($\chi^2=8.322$; $P<0.01$). However, there is no statistically significant difference in the prevalence of CMV infection between genders.

The identification of patients at high risk for CMV replication according to the serostatus of donor and recipients provides the rationale for prophylactic and preemptive administration of antivirals and significantly reduces CMV disease and associated morbidity (Egli et al., 2007).

Serological screening for CMV antibodies should be performed on both donors and recipients before transplantation to identify patients at risk for infection after transplantation who might benefit from a preventive strategy. CMV prevention must be individualized by risk group and immunosuppressive regimen. Patients at risk for primary infection (D+/R-) are generally given valganciclovir prophylaxis for 3-6 months after transplantation, while 6 months of prophylaxis is often used in patients (D+/R- or R+) receiving depleting antilymphocyte antibodies (Taber et al., 2004). Patients at lower risk (R+) may also be followed-up using quantitative assays at predefined intervals (weekly) to detect and treat early disease (preemptive therapy). In renal transplant recipients receiving either prophylaxis (oral ganciclovir) or weekly PCR surveillance and treatment for either a positive assay or invasive disease, using prophylaxis was more effective at preserving renal function and had the lowest risk of rejection (Geddes et al., 2003). Two main strategies are employed to prevent CMV. The first, so-called universal prophylaxis (to prevent infection before its onset), is used for all patients at risk of disease (D+/R+) and is continued for a set period of time. The second, known as targeted prophylaxis, focuses its strategies on those at highest risk for the disease (D+/R-). An alternative to prophylaxis is a preemptive strategy that employs periodic screening for occult CMV viremia and initiation of early treatment (Green et al., 2004).

EBV has a central role in the pathogenesis of post-transplant lymphoproliferative disease (PTLD), although not all PTLD are EBV-related (Preiksaitis

and Keay, 2001). Under conditions of severe T cell immunosuppression, which dominate in transplant patients, EBV-infected B cells may expand unrestricted, resulting in malignant lymphoproliferation. In this context, the virus is able to transform and immortalize B-lymphocytes, leading to their uncontrolled proliferation. This is particularly likely in settings where the host lacks adequate cytotoxic T lymphocyte surveillance. One such setting occurs when transplant recipients experience primary EBV infection. The majorities of these tumors, known collectively as B cell lymphoproliferative disorders or post transplantation lymphoproliferative disease, when present in transplant recipients, are EBV-associated and often occur within the first year following transplantation (Allen et al., 2002).

Primary EBV infection is a major risk factor for the development of these tumors; however, PTLD has also been documented in patients with reactivated infection. Thus, the incidence of PTLD is greater in children than in adults, with rates of 4% to 22% reported for the various categories of pediatric organ transplant recipients versus an average of 1% to 2% in adults (Green, 2001).

In this study, EBV DNA was obtained and detected only in the pediatric renal transplant recipients. Four of 49 (8.16%) pediatric kidney transplant recipients had an EBV-positive finding in the tested plasma samples. EBV DNA was not detected in the tested samples obtained from adult renal transplant recipients.

This difference between adults and children is understandable in view of the fact that in EBV-seronegative transplant recipients EBV infection rates are high, approaching 100% within three months after transplantation (Allen et al., 2001). Children are more likely to contract a primary EBV infection after transplantation, usually from the donor organ or blood transfusions (Savoie et al., 1994; Ho et al., 1988), because many are EBV-seronegative before transplantation. In developed countries, EBV seropositivity rates increase with age; therefore, in the general population of children from 1 to 18 years of

age, up to 50% are EBV-seropositive, compared with a 90% seropositivity rate in adults (Allen et al., 2002; Burra et al., 2006).

EBV serology is important for pre-transplantation identification of the risk factors for PTLD (e.g. the identification of D+R- subjects). Knowing this, the risk of PTLD may decrease if it were possible to give EBV-seronegative recipients EBV-seronegative organs. However, the form of donor-recipient matching is often not a practical preventive strategy, because of the limited supply of donor organs (Allen et al., 2002). Serology has limited or no value after transplantation unless it is part of an evaluative study. The main component of laboratory surveillance for EBV-related diseases after transplantation revolves around the use of sequential PCR testing to document the presence of infection and the magnitude of EBV load. Serological tests are of limited value (Tsai et al., 2002).

The human polyomavirus BKV has only recently surfaced as a pathogen with organ tropism largely limited to the renourinary tract, manifesting as a polyomavirus-associated nephropathy in kidney transplant recipients. Transmission of BKV occurs typically during childhood (median 4-5 years of age) via oral and respiratory routes, but data suggesting transmission via cells and tissues, in particular by kidney transplantation, have been reported (Knowles et al., 2003). Seroprevalence increases more than 80% in adults (Hirsch et al., 2006). After primary replication in seronegative individuals, BKV establishes non-replicative infection in the renourinary system, without known complications for the immunocompetent host. In about 5% of healthy individuals, intermittently reactivation with detectable viruria of BKV occurs (Dolei et al., 2000).

Polyomavirus associated nephropathy (PVAN) and late-onset hemorrhagic cystitis are major complications linked to high-level BKV replication in kidney transplant recipients (Allander et al., 2007). Recently many investigators have studied the important role of BKV in PVAN. PVAN complicates kidney transplantation in 1-10% of cases, usually at the end

of the first year post-transplantation, with clinically silent, creeping allograft failure in 50-90%. Graft loss may occur in around 50% of cases during the subsequent 6-60 months. Persisting BKV replication is associated with a higher probability of graft loss.

According to the results of this study, the prevalence of BKV (32.65%) is much higher than the prevalence of JCV (9.68%) in immunocompromised patients. Although viremia of both polyomaviruses is more frequent in kidney recipients than viremia, results from this study did not show a statistically significant difference between polyomaviruses viremia and viremia (12.9% and 8%, respectively).

Therefore, testing for the presence of BKV DNA in the urine has become the most pivotal test to exclude PVAN in 65-85% of kidney transplant patients, whereas in patients with detectable viremia, plasma BKV loads allow the diagnosis of "presumptive PVAN" in cases with confirmed higher values to the equivalent of >10 000 copies/ml. Screening for BKV DNA is therefore recommended every 3 months during the first 2 years post-transplantation, when allograft biopsies are performed for any reason, or when allograft dysfunction occurs (Hirsch et al., 2005). Currently, reducing immunosuppression is considered to be the intervention of choice. The absence of effective anti-polyomaviral drugs renders BKV treatment strategies largely dependent on the immunological containment of BKV replication. Assays quantifying virus-specific cellular immune responses in real-time are important new avenues to be explored to better predict the risk of replication and disease and to optimize clinical management (Hirsch, 2006).

CONCLUSION

The study of viral infectious diseases associated with transplantation focuses on the prevention of infection in transplant recipients. The interaction between infection and immunosuppression after transplantation has a crucial role. The induction of immunological tolerance might, if successful, reduce the risk of viral infection after transplantation. However,

two caveats remain. Firstly, exposures to infections subsequent to the development of tolerance might abrogate tolerance and induce allograft rejection. Secondly, the induction of tolerance to an allograft might induce immunologic unresponsiveness to latent microorganisms in the organ.

Techniques currently under development, including more sensitive microbiology assays and immunoassays, can provide the potential for individualized immunosuppression and prophylactic strategies. Such assays may ultimately permit a more dynamic assessment of the immune status of transplant recipients over time, allowing titration of immunosuppression and reducing serious illness or death from viral infection and/or malignant conditions.

Acknowledgments - This study was supported by Ministry of Science and Environmental Protection, Republic of Serbia, research Grants 175073, 175038 and 175089.

REFERENCES

- Allander, T., Andreasson, K., Gupta, S., Bjerkner, A., Bogdanovic, G., Persson, M.A., Dalianis, T., Ramqvist, T., and B. Andersson (2007). Identification of a third polyomavirus. *J. Virol.* **81**, 4130-4136.
- Allen, U., Alfieri, C., Preiksaitis, J., Humar, A., Moore, D., Tapiero, B., Tellier, R., Green, M., Davies, D., Hébert, D., Weitzman, S., Petric, M., Jacobson, K., and Canadian PTLD Workshop Group (2002). Epstein-Barr virus infection in transplant recipients: Summary of a workshop on surveillance, prevention and treatment. *Can. J. Infect. Dis.* **13**(2), 89-99.
- Allen, U., Hebert, D., Petric, M., Tellier, R., Tran, D., Superina, R., Stephens, D., West, L., Wasfy, S., and S. Nelson (2001). Utility of semiquantitative polymerase chain reaction for Epstein-Barr virus among pediatric solid organ transplant recipients with and without post-transplant lymphoproliferative disease. *Clin. Infect. Dis.* **33**, 145-150.
- Babel, N., Schwarzmann, F., Prang, N., Jaeger, M., Wolf, H., Kern, F., Volk, H.D., and P. Reinke (2001). Association between Epstein-Barr virus infection and late acute transplant rejection in long-term transplant patients. *Transplantation.* **72**, 736-739.
- Barzon, L., Murer, L., Pacenti, M., Biasolo, M.A., Della Vella, M., Benetti, E., Zanon, G.F., and G. Palù (2009). Investigation of intrarenal viral infections in kidney transplant recipients unveils an association between parvovirus B19 and chronic allograft injury. *J. Infect. Dis.* **199**, 372-380.

- Barzon, L., Murer, L., Pacenti, M., Biasolo, M.A., Della Vella, M., Ghirard, G., Gamba, P.G., De Arias, A.E., Zanon, G.F., and G. Palu (2009). Detection of viral DNA in kidney graft preservation and washing solutions is predictive of post-transplant infections in pediatric recipients. *J. Infect. Dis.* **200**, 1425-1433.
- Boeckh, M., and W.G. Nichols (2003). Immunosuppressive effects of beta-herpesviruses. *Herpes*. **10**, 12-16.
- Brennan, D.C. (2001). Cytomegalovirus in renal transplantation. *J. Am. Soc. Nephrol.* **12**, 848-855.
- Buehrig, C.K., Lager, D.J., Stegall, M.D., Kreps, M.A., Kremers, W.K., Gloor, J.M., Schwab, T.R., Velosa, J.A., Fidler, M.E., Larson, T.S., and M.D. Griffin (2003). Influence of surveillance renal allograft biopsy on diagnosis and prognosis of polyomavirus-associated nephropathy. *Kidney Int.* **64**, 665-673.
- Burra, P., Buda, A., Livi, U., Rigotti, P., Zanusi, G., Calabrese, F., Caforio, A., Menin, C., Canova, D., Farinati, F., and S.M. Aversa (2006). Occurrence of post-transplant lymphoproliferative disorders among over thousand adult recipients: any role for hepatitis C infection? *Eur. J. Gastroenterol. Hepatol.* **18**(10), 1065-1070.
- Castagnola, E., Faraci, M., Moroni, M., Di Marco, E., Cirillo, C., Rabagliati, A.M., Ricci, R., Natalizia, A.R., de Fazio, V., Morreale, G., Granata, C., Lanino, E., Dini, G., and R. Haupt (2008). Rare viral infections in children receiving hemopoietic stem cell transplant. *Bone Marrow Transplantation* **41**, S100-S103.
- Djamali, A., Samaniego, M., Muth, B., Muehrer, R., Hofmann, M., Pirsch, J., Howard, A., Mourad, G., and B.N. Becker (2006). Medical Care of Kidney Transplant Recipients after the First Posttransplant Year. *Clin. J. Am. Soc. Nephrol.* **1**, 623-640.
- Dockrell, D.H., and C.V. Paya (2001). Human herpesvirus-6 and -7 in transplantation. *Rev. Med. Virol.* **11**, 23-36.
- Dolei, A., Pietropaolo, V., Gomes, E., Di Taranto, C., Ziccheddu, M., Spanu, M.A., Lavorino, C., Manca, M., and A.M. De-gener (2000). Polyomavirus persistence in lymphocytes: prevalence in lymphocytes from blood donors and healthy personnel of a blood transfusion centre. *J. Gen. Virol.* **81**, 1967-1973.
- Egli, A., Binggeli, S., Bodaghi, S., Dumoulin, A., Georg, A., and F.N. Khanna (2007). Cytomegalovirus and polyomavirus BK posttransplant. *Nephrol. Dial. Transplant.* **22** (Suppl.8) 72-82.
- Fishman, J.A. (2007). Infection in solid-organ transplant recipients. *N. Engl. J. Med.* **357**, 2601-2614.
- Geddes, C.C., Church, C.C., Collidge, T., McCrudden, E.A., Gillespie, G., Matthews, E., Hainmueller, A., and J.D. Briggs (2003). Management of cytomegalovirus infection by weekly surveillance after renal transplant: Analysis of cost, rejection and renal function. *Nephrol. Dial. Transplant.* **18**, 1891-1898.
- Ginevri, F., De Santis, R., Comoli, P., Pastorino, N., Rossi, C., Botti, G., Fontana, I., Nocera, A., Cardillo, M., Ciardi, M.R., Locatelli, F., Maccario, R., Perfumo, F., and A. Azzi (2003). Polyomavirus BK infection in pediatric kidney-allograft recipients: a single-center analysis of incidence, risk factors, and novel therapeutic approaches. *Transplantation*, **75**, 1266-1270.
- Green, M. (2001). Management of Epstein-Barr virus-induced posttransplant lymphoproliferative disease in recipients of solid organ transplantation. *Am. J. Transplant.* **1**, 103-108.
- Green, M., Avery, R., and J. Preiksaitis (2004). Guidelines for the prevention and management of infectious complications of solid organ transplantation. *Am. J. Transplant.* **4** [Suppl 10], 10-17, 57-109, 160-163.
- Green, M., Michaels, M.G., Katz, B.Z., Burroughs, M., Gerber, D., Shneider, B.L., Newell, K., Rowe, D., and J. Reyes (2006). CMV-IVIG for prevention of Epstein Barr virus disease and posttransplant lymphoproliferative disease in pediatric liver transplant recipients. *Am. J. Transplant.* **6**, 1906-1912
- Hartmann, A., Sagedal, S., and J. Hjelmestaeth (2006). The natural course of cytomegalovirus infection and disease in renal transplant recipients. *Transplantation*, **82** [Suppl], S15-S17.
- Hirsch, H.H., Brennan, D.C., Drachenberg, C.B., Ginevri, F., Gordon, J., Limaye, A.P., Mihatsch, M.J., Nickeleit, V., Ramos, E., Randhawa, P., Shapiro, R., Steiger, J., Suthanthiran, M., and J. Trofe (2005). Polyomavirus-associated nephropathy in renal transplantation: interdisciplinary analyses and recommendations. *Transplantation*, **79**, 1277-1286.
- Hirsch, H.H., Drachenberg, C.B., Steiger, J., and E. Ramos (2006). Polyomavirus-associated nephropathy in renal transplantation: critical issues of screening and management. *Adv. Exp. Med. Biol.* **577**, 160-173.
- Hirsch, H.H., Rinaldo, C.H., Drachenberg, C.D., Ramos, E., Steiger, J., and R. Gosert (2006). Emergence of rearrangements in the BKV non-coding control region in renal transplant patients (Abstract 73). *Am. J. Transplant.* **6**, [S2] 91.
- Ho, M., Jaffe, R., Miller, G., Breinig, M.K., Dummer, J.S., Makowska, L., Atchison, R.W., Karrer, F., Nalesnik, M.A., and T.E. Starzl (1988). The frequency of Epstein-Barr virus infection and associated lymphoproliferative syndrome after transplantation and its manifestations in children. *Transplantation*, **4**, 719-727.
- Knowles, W.A., Pipkin, P., Andrews, N., Vyse, A., Minor, P., Brown, D.W., and E. Miller (2003). Population-based

- study of antibody to the human polyomaviruses BKV and JCV and the simian polyomavirus SV40. *J. Med. Virol.* **71**, 115-123.
- Kidd, I.M., Clark, D.A., Sabin, C.A., Andrew, D., Hassan-Walker, A.F., Sweny, P., Griffiths, P.D., and V.C. Emery (2000). Prospective study of human betaherpesviruses after renal transplantation: Association of human herpesvirus 7 and cytomegalovirus co-infection with cytomegalovirus disease and increased rejection. *Transplantation.* **69**, 2400-2404. *J. Transplant.* **4**, 378-383.
- Opelz, G., Naujokat, C., Daniel, V., Terness, P., and B. Dohler (2006). Disassociation between risk of Kotton, C.N., and J.A. Fishman (2005). Viral Infection in the Renal Transplant Recipient. *J. Am. Soc. Nephrol.* **16**, 1758-1774.
- Liapis, H., Storch, G., Hill, D.A., Rueda, J., and D. Brennan (2003). CMV infection of the renal allograft is much more common than the pathology indicates: A retrospective analysis of qualitative and quantitative buffy coat CMV-PCR, renal biopsy pathology and tissue CMV-PCR. *Nephrol. Dial. Transplant.* **18**, 397-402.
- Ljungman, P. (2002). Beta-herpesvirus challenges in the transplant recipient. *J. Infect. Dis.* **186** [Suppl 1], S99-S109.
- Meier-Kriesche, H.U., Schold, J.D., Srinivas, T.R., and B. Kaplan (2004). Lack of improvement in renal allograft survival despite a marked decrease in acute rejection rates over the most recent era. Am. graft loss and risk of non-Hodgkin lymphoma with induction agents in renal transplant recipients. *Transplantation*, **81**, 1227-1233.
- Paya, C.V., Humar, A., Dominguez, E., Washburn, K., Blumberg, E., Alexander, B., Freeman, R., Heaton, N., Pescovitz, M.D., and Valganciclovir Solid Organ Transplant Study Group: Efficacy and safety of valganciclovir vs. oral ganciclovir for prevention of cytomegalovirus disease in solid organ transplant recipients. *Am. J. Transplant.* **4**, 611-620
- Randhawa, P., and D.C. Brennan (2006). BK virus in transplant recipients: An overview and update. *Am. J. Transplant.* **6**, 2000-2005.
- Razonable, R.R., Rivero, A., Brown, R.A., Hart, G.D., Espy, M.J., van Crujnsen, H., Wilson, J., Groettum, C., Kremers, W., Smith, T.F., and C.V. Paya (2003). Detection of simultaneous beta-herpesvirus infections in clinical syndromes due to defined cytomegalovirus infection. *Clin. Transplant* **17**, 114-120.
- Rubin, R.H. (2002). The direct and indirect effects of infection in liver transplantation: Pathogenesis, impact, and clinical management. *Curr. Clin. Top. Infect. Dis.* **22**, 125-154.
- Sageda, S., Nordal, K.P., Hartmann, A., Sund, S., Scott, H., Degre, M., Foss, A., Leivestad, T., Osnes, K., Fauchald, P., and H. Rollag (2002). The impact of cytomegalovirus infection and disease on rejection episodes in renal allograft recipients. *Am. J. Transplant.* **2**, 850-856.
- Savoie, A., Perpète, C., Carpentier L., Joncas, J., and C. Alfieri (1994). Direct correlation between the load of Epstein-Barr virus-infected lymphocytes in the peripheral blood of pediatric transplant patients and risk of lymphoproliferative disease. *Blood.* **83**, 2715-2722.
- Taber, D.J., Ashcraft, E., Baillie, G.M., Berkman, S., Rogers, J., Baliga, P.K., Rajagopalan, P.R., Lin, A., Emovon, O., Afzal, F., and K.D. Chavin (2004). Valganciclovir prophylaxis in patients at high risk for the development of cytomegalovirus disease. *Transpl. Infect. Dis.* **6**, 101-109.
- Tong, C.Y., Bakran, A., Williams, H., Cheung, C.Y., and J.S. Peiris (2000). Association of human herpesvirus 7 with cytomegalovirus disease in renal transplant recipients. *Transplantation*, **70**, 213-216.
- Toyoda, M., Puliyaanda, D., Amet, N., Baden, L., Cam, V., Radha, R., Pao, A., Vo, A., Bunnapradist, S., Moudgil, A., and S. Jordan (2005). Co-infection of polyomavirus-BK and cytomegalovirus in renal transplant recipients. *Transplantation*, **80**, 198-205.
- Tsai, D.E., Nearey, M., Hardy, C.L., Tomaszewski, J.E., Kotloff, R.M., Grossman, R.A., Olthoff, K.M., Stadtmauer, E.A., Porter, D.L., Schuster, S.J., Luger, S.M., and R.L. Hodinka (2002). Use of EBV PCR for the diagnosis and monitoring of post-transplant lymphoproliferative disorder in adult solid organ transplant patients. *Am. J. Transplant*, **2**(10), 946-954.
- Vasudev, B., Hariharan, S., Hussain, S.A., Zhu, Y.R., Bresnahan, B.A., and E.P. Cohen (2005). BK virus nephritis: risk factors, timing, and outcome in renal transplant recipients. *Kidney Int.* **68**, 1834-1839.
- Weikert, B.C., and E.A. Blumberg (2008). Viral Infection after Renal Transplantation: Surveillance and Management. *Clin. J. Am. Soc. Nephrol.* **3**, S76-S86.
- Zhang, L.G., Hanpf, P., Rutherford, C., Churchill, W.H., and C.S. Crumacker (1995). Detection of human cytomegalovirus DNA, RNA, and antibody in normal donor blood. *J. Infect. Dis.* **171**, 1002-1006.