Medicinski podmladak

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ORIGINAL ARTICLE

# THE PREVALENCE OF HERPESVIRUS INFECTIONS IN CHILDREN AND YOUNG ADULTS TRANSPLANT RECIPIENTS - KIDNEY AND HEMATOPOETIC STEM CELLS

## *PREVALENCIJA HERPESVIRUSNIH INFEKCIJA KOD PEDIJATRIJSKIH PACIJENATA POSLE TRANSPLANTACIJE BUBREGA I MATIČNIH ĆELIJA KOŠTANE SRŽI*

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Abstract

**Introduction:** Viruses are the most important and common cause of opportunistic infections following transplantation. The risk correlates with the virus encountered, transplanted tissue and organ, intensity of immune suppression, and other host factors governing susceptibility. Infections caused by the human herpesviruses, continue to challenge the clinical management of transplant recipients.

**Aim:** The aim of this study was to investigate the prevalence of herpesvirus infections among pediatric hematopoetic stem cell and renal transplant recipients (HSCTR and RTR).

**Material and methods:** This is a retrospective study of 150 pediatric HSCTR and RTR investigated in plasma samples by PCR in multiple testings, on the presence of cytomegalovirus (CMV), Epstein–Barr virus (EBV), herpes simplex virus type 1 and 2 (HSV1/2) and human herpes virus 6 (HHV6) during 2015/2016 period. Visualization of PCR products was performed by electrophoresis on 2% agarose gel with ethidium bromide. For statistical analyses T test, McNemar's test, Chi - square and Fisher's exact test were used.

**Results:** During 2015, statistical significance was reached at the follow ups, where 33.3% (p=0.031) and 46.7% (p=0.016) of HSCTR, and 4.3% and 28.0% of RTR, had positive CMV and EBV results, respectively, in regard to the first test. During 2016, similar finding was observed where HSCT recipients had 70.6% CMV (p=0.002) and 29.4% EBV positive results during the follow ups. Cytomegalovirus (CMV) finding was negative in all RTR, but 12.5 and 4.0% of investigated kidney recipients were EBV positive during the first test and follow ups, respectively.

**Conclusion:** The results demonstrated that HSCTR are in a greater risk of CMV and EBV infections, compared to RTR. Therefore, the importance of permanent post - transplant monitoring of herpesviruses is in timely diagnosis and prevention of overt infections from occurring.

**Key words:**
herpesviruses,
pediatric transplant recipients,
HSCTR,
RTR,
post-transplant CMV monitoring,
post-transplant EBV monitoring



The authors declare no conflicts of interest.

doi:10.5937/mp69-16747
Editorial board: podmladak.med.bg@gmail.com

Sažetak

**Uvod:** Virusi su najznačajniji i najčešći uzročnici oportunističkih infekcija nakon transplantacije. Rizik od nastanka virusnih infekcija korelira sa vrstom virusa, tipom transplantiranog tkiva i organa, vrstom i dozom imunosupresiva, stepenom imunosupresije i drugim faktorima koji povećavaju osjetljivost recipijenata za nastanak infekcije. Herpes virusne infekcije predstavljaju kontinuirani rizik kod primaoca solidnih organa, odnosno matičnih ćelija hematopoeze.

**Cilj:** Cilj rada je bio da se ispita prevalencija herpes virusa kod djece posle transplantacije matičnih ćelija hematopoeze (HSCTR) i bubrega (RTR).

**Materijal i metode:** Ovo je retrospektivna studija koja je izvedena tokom 2015-2016. godine i obuhvatila je 150 pedijatrijskih HSCTR i RTR, čiji su uzorci plazme višekratno testirani na prisustvo citomegalovirusa (CMV), Epštajn-Bar virusa (Epstein Barr, EBV), herpes simpleks virusa 1, 2 (HSV1/2) i humanog herpes virusa 6 (HHV6) metodom lančane reakcije polimeraze (PCR).Vizualizacija PCR proizvoda vršena je metodom elektroforeze u 2% agaroznom gelu pomoću etidijum-bromida. Za statističku analizu korišćeni su T-test, Meknemar (McNemar), Hi kvadrat i Fišerov test.

**Rezultati:** Tokom 2015. godine statistička značajnost je uočena u kontrolnim nalazima, gdje je 33,3% HSCTR (p = 0,031) imalo pozitivan CMV, odnosno 46,7% (p = 0,016) pozitivan EBV u odnosu na prvo testiranje. Među RTR, 4,3% je imalo pozitivan CMV, a 28,0% EBV pozitivan nalaz u ponovnom testiranju. U 2016. godini uočen je sličan nalaz, gdje je 70,6% (p = 0,002) recipijenata koštane srži imalo pozitivan CMV, odnosno 29,4% pozitivan EBV u kontrolnim nalazima, dok je CMV bio negativan kod svih RTR i u prvom i u ponovnom testiranju, a EBV je bio pozitivan kod 12,5% pacijenata u prvom, odnosno 4,0% u kontrolnim testiranjima.

**Zaključak:** Na osnovu dobijenih rezultata, HSCTR su u većem riziku za nastanak CMV i EBV infekcija od RTR. Permanentni monitoring herpes virusa tokom posttransplantacionog perioda značajan je za pravovremeno postavljanje dijagnoze i prevenciju nastanka manifestnih infekcija.

**Ključne reči:**
herpes virusi,
djeca primaoci bubrega,
djeca primaoci koštane srži,
posttransplantacioni
CMV monitoring,
posttranplantacioni EBV
monitoring

## Introduction

The number of Hematopoetic Stem Cell Transplant (HSCTR), Bone Marrow recipients (BMR) and Renal Transplant recipients (RTR) are increasing, with lifestyle and life expectancy approaching non - transplanted persons (1).

The objective of HSCT is to replace the hematopoetic and lymphoid system of a patient, which can be done through two processes. First process is an autologous HSCT, where the patient's own cells are sampled and reinfused after total body irradiation (TBI) or aggressive chemotherapy treatment, with the purpose of completely eliminating the patient's hematopoetic stem cells. Second process is an allogeneic HSCT, where the patient receives cells from another individual, to whom the patient can be related or not. Indications for these procedures vary and include neoplastic diseases, congenital immunodeficiencies, acute leukemias, etc (2). The risk of infection after allogeneic HSCT is considerably higher than autologous HSCT, and is further alleviated by most severe complications of this procedure (graft rejection, Graft versus Host Disease, etc) (3).

Kidney transplantation is the most promising treatment for patients with terminal kidney failure, resulting in longer life expectancy and better quality of life (4). Indications for pediatric renal transplantation include delayed psychomotor development, Rickets, persistent electrolyte imbalances, etc (5). Immunosuppressive therapy in these patients brings an array of adverse effects which affect patients' survival and quality of life (6). Development of more effective immunosuppression protocols decreased the incidence of acute rejection, while at the same time increased the incidence of viral infections (7). Viral infections in the post - transplant period are associated with higher morbidity, even mortality (8).

Transplant patients are submitted to immunosuppressive therapy that profoundly impairs T - lymphocyte function. These patients are particularly susceptible to diseases caused by the relevant herpesviruses such as CMV, EBV, HSV1/2 and HHV6 (9). In transplanted patients, HSV1/2 cause extensive and severe muco - cutaneous infections, but the most severe and important infection is encephalitis. Cytomegalovirus, while causing mild infections in immunocompetent individuals, in transplanted patients can cause an infectious syndrome of life threatening severity. Lymphoproliferative syndrome is a hallmark of EBV infection in immunocompromised, while HHV6 is associated with rejection in transplanted kidneys, often as a cofactor with CMV or EBV (10).

The aim of this paper was to investigate the prevalence of herpesvirus infections among pediatric hematopoetic stem cell and kidney transplant recipients.

## Material and Methods

### Patients

Children, both HSCTR and RTR, from the Institute for Health Protection of Mother and Child of Serbia "Dr. Vukan Cupic" and University Children's Hospital, Belgrade, Serbia, were retrospectively reviewed in the period from January 2015 to December 2016. Plasma samples were tested for the presence of relevant herpesviruses: HSV1/2, CMV, EBV and HHV6 in the Virology Laboratory of Institute of Microbiology and Immunology, Faculty of Medicine, University of Belgrade. A total of 150 bone marrow and renal transplant recipients were included in this study (HSCTR boys 25, median age 10; range 1-17 and 19 girls, median age 10; range 1-15; RTR boys 64, median age 18; range 2-22 and 43 girls, median age 16; range 2-22). All four relevant herpes viruses were tested in blood samples of HSCT recipients, whereas only CMV and EBV were tested in blood samples of RT recipients, according to the protocols.

Isolation and amplification of viral DNA

All 150 patients were tested for herpesvirus DNA in plasma samples, by PCR-based test. Due to variable clinical course, our patients underwent subsequent control tests after the initial virology test. Blood samples were collected in 5mL Vacutainer - tubes containing EDTA. The DNA extraction was performed from 200 μL of plasma using QIAmp Blood Mini Kit (QiAGEN GmBH, Hilden, Germany), according to the manufacturer's protocols. Blood samples were most frequently tested at least two times, to prove the presence of relevant herpes viruses, with a time interval of 7 – 30 days between two testing. In this way, monitoring of herpesvirus infections was done with patients who had a positive result and received therapy.

Using the qualitative commercial End - point kit HSV 430/720IC (Sacace, Biotechnologies S.r.l., Como, Italy), HSV1/2 DNA was detected, according to the manufacturer's instruction. For nucleic acid amplification of DNA specific primers were used, directed against glycoprotein B - gene of HSV. The length of specific amplified HSV DNA fragment is 430 bp.

Using the PCR targeting of a 435 bp region in the exon 4 of Mayor Immediate Early (MIE) gene, CMV DNA was amplified. The amplification protocol was performed using primers: FW 5'-ccaagcggcctctgataaccaagcc-3' and REV 5'-cagcaccatcctcctcttcctctgg -3' following several steps: an initial denaturation at 950C for 5 min, followed by 35 cycles 950C - 60sec, 580C - 60sec, 720C – 90sec, and terminal elongation at 720C - 10 min (11).

For EBV DNA detection by nested PCR, two different sets of primers were used. The first-outer set amplified a 663 bp (FW 5'-gctaaggcattcccagtaaa-3'; REV 5'-gatgaacaccaccacgatg-3') and the second-inner set of primers (FW 5'-cggaaccagaagaccca-3'; REV 5'-tcccgcaccctcaacaag-3') amplified a 506 bp within Carboxyl terminal LMP 1 coding region of EBV genome. The program for both PCR reactions has initial denaturation step at 95˚C for 5 minutes, 40 cycles of denaturation at 95˚C for 60 seconds, annealing at 47˚C for 60 seconds and extension at 72˚C for 1 minutes with the final extension step of 72˚C for 10 minutes (12).

The 520 bp fragment within UL67 coding region of HHV6 genome was amplified using primers: FW (5'-gcgttttcagtgtgtagttcggcag- 3') and REV (5'-tggccgcattcgtacagatacggagg-3'). The PCR program, for amplification of HHV6, was initial denaturation at 95˚C for 5 minutes, 57˚C and 72˚C 1 min, 30 cycles of denaturation at 94˚C for 60 seconds, annealing at 58˚C for 60 seconds and extension at 72˚C for 1 minute with the final extension step of 72˚C for 10 minutes (13).

The amplification of PCR, for all examined viral DNA, was run in a reacting volume of 25 μL containing 12,5μL PCR Master Mix (QIAgenTaq PCR Master Mix, Hilden Germany) 1μL (1μM) FW and 1μL (1μM) REV primers, 5μL previously isolated DNA and 5,5μL injection grade water. The PCR was carried out in thermocycler Master Cycler Gradient (Eppendorf, Germany).

Visualization of PCR products

Visualization of all PCR products of appropriate length was performed by electrophoresis in 2% agarose gel stained with ethidium bromide and 100bp DNA standard.

Statistical analyses

For statistical analyses T test for two independent samples, McNemar's test, Chi – square test and Fisher's exact test were used. All p values < 0,05 were considered statistically significant and p values < 0,001 were considered highly statistically significant.

## Results

The study included a total of 150 transplanted patients, which were post - transplantation tested on four most relevant herpesviruses (CMV, EBV, HSV1/2 and HHV6), during the whole study period, ie. 68 (45.3%) during 2015 and 82 (54.7%) during 2016. During 2015, 20 (29.4%) of the HSCTR and 48 (70.6%) of RTR were tested for the herpesviruses, and in 2016, 23 (28.0%) of the HSCTR and 59 (72.0%) of RTR were tested for the same viruses.

In regard to the number of patients tested, there was no statistically significant difference ($p = 0.253$, 68 vs. 82), however statistically highly significant more frequent tests were done among children with kidney transplant ($p < 0.001$, HSCT vs. RT = 43 vs. 107). This difference was also observed within both years of our study. (**Diagram 1**)

Pediatric HSCTR were, according to statistical analysis, highly significantly younger than RTR in each year of the study - 10.55 vs. 15.44 in 2015 ($p < 0.001$) and 9.18 vs. 15.34 in 2016 ($p < 0.001$), respectively. (**Table 1**)

The most frequently tested herpesviruses were CMV and EBV, and these tests came out with mostly negative results, both on the first test and in subsequent repeated testing. However, comparing the HSCTR first testing

to their follow ups, it can be seen that they had a greater number of positive results during the follow ups, in comparison with their first tests. (**Table 2 and Table 3**)

In 2015, all patients tested had negative HSV1/2 and HHV6 results both at the first testing and the follow ups, whereas during 2016, a significant number of positive results was noticed at the follow ups. (**Table 2 and Table 3**)

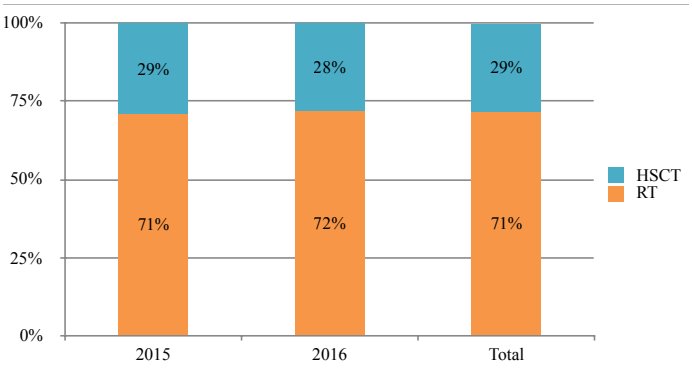


**Diagram 1.** Comparison of number of tested children during 2015 and 2016.

**Table 1**. Average age of pediatric renal and HSCT patients during 2015 and 2016.

| Year | Transplant Type | x ±sd | p§ |
|---|---|---|---|
| 2015 | Hematopoietic stem cell transplant recipients | 10.55±4.27 | <0.001 |
| | Renal transplant recipients | 15.44±5.24 | |
| 2016 | Hematopoietic stem cell transplant recipients | 9.18±5.57 | <0.001 |
| | Renal transplant recipients | 15.34±5.74 | |

**Table 2**. Results of all tests during 2015.

| 2015 | Transplant type | | CMV | EBV | HSV1/2 | HHV6 |
|---|---|---|---|---|---|---|
| **First Test** | Hematopoietic stem cell transplant | negative | 20 (100%) | 19 (95.0%) | 2 (100%) | 2 (100%) |
| | | positive | 0 (0%) | 1 (5.0%) | 0 (0%) | 0 (0%) |
| | Renal transplant | negative | 45 (100%) | 39 (83.0%) | N/A | N/A |
| | | positive | 0 (0%) | 8 (17.0%) | | |
| **Follow Ups** | Hematopoietic stem cell transplant | negative | 10 (66.7%) | 8 (53.3%) | 5 (100%) | 2 (100%) |
| | | positive | 5 (33.3%) | 7 (46.7%) | 0 (0%) | 0 (0%) |
| | Renal transplant | negative | 22 (95.7%) | 18 (72.0%) | N/A | N/A |
| | | positive | 1 (4.3%) | 7 (28.0%) | | |

**Table 3**. Results of all tests during 2016.

| 2016 | Transplant type | | CMV | EBV | HSV1/2 | HHV6 |
|---|---|---|---|---|---|---|
| **First Test** | Hematopoietic stem cell transplant | negative | 21 (95.5%) | 20 (95.2%) | 9 (100%) | 9 (100%) |
| | | positive | 1 (4.5%) | 1 (4.8%) | 0 (0%) | 0 (0%) |
| | Renal transplant | negative | 54 (100%) | 49 (87.5%) | N/A | N/A |
| | | positive | 0 (0%) | 7 (12.5%) | | |
| **Follow Ups** | Hematopoietic stem cell transplant | negative | 5 (29.4%) | 12 (70.6%) | 7 (53.8%) | 13 (92.9%) |
| | | positive | 12 (70.6%) | 5 (29.4%) | 6 (46.2%) | 1 (7.1%) |
| | Renal transplant | negative | 25 (100%) | 24 (96.0%) | N/A | N/A |
| | | positive | 0 (0%) | 1 (4.0%) | | |

Statistical results showed that at the first testing there was no difference among pediatric HSCTR, compared to RTR, in the prevalence of CMV and EBV during the whole study period ($p = 0.298$, $p = 0.104$). (**Table 4**) However, there was a statistically significant difference at the follow ups for the same study period ($p < 0.001$, $p = 0.027$). (**Table 5**)

Furthermore, the prevalence of HSCTR positive CMV results at the first test is 2.4%, whereas at the follow ups 53.1%. None of RTR had a positive CMV result at the first test, and 2.1% had at the follow ups. Regarding EBV, the prevalence of positive results among HSCTR at the first test was 4.9%, and in subsequent follow ups 37.5%, and the prevalence of RTR positive results was 14.6% during the first test and 16.0% at the follow ups. (**Table 4** and **Table 5**)

**Table 4.** Comparison of EBV and CMV results between HSCT recipients and RT recipients at the first testing during the whole study period.

| Virus | Result | HSCT recipients n, % | RT recipients n, % | p* |
|---|---|---|---|---|
| **CMV** | Positive | 1 (2.4) | 0 (0.0) | 0.298¥ |
| | Negative | 41 (97.6) | 99 (100.0) | |
| **EBV** | Positive | 2 (4.9) | 15 (14.6) | 0.104§ |
| | Negative | 39 (95.1) | 88 (85.4) | |

**Table 5.** Comparison of EBV and CMV results between HSCT recipients and RT recipients at the follow ups during the whole study period.

| Virus | Result | HSCT recipients n, % | RT recipients n, % | p* |
|---|---|---|---|---|
| **CMV** | Positive | 17 (53.1) | 1 (2.1) | <0.001§ |
| | Negative | 15 (46.9) | 47 (97.9) | |
| **EBV** | Positive | 12 (37.5) | 8 (16.0) | 0.027§ |
| | Negative | 20 (62.5) | 42 (84.0)) | |

The prevalence of positive CMV and EBV results among both HSCTR and RTR was greater at the follow ups during 2015. (**Diagram 2**)

Moreover, during 2016 the prevalence of positive results was greater within both groups of patients during the follow ups, as well. (**Diagram 3**)

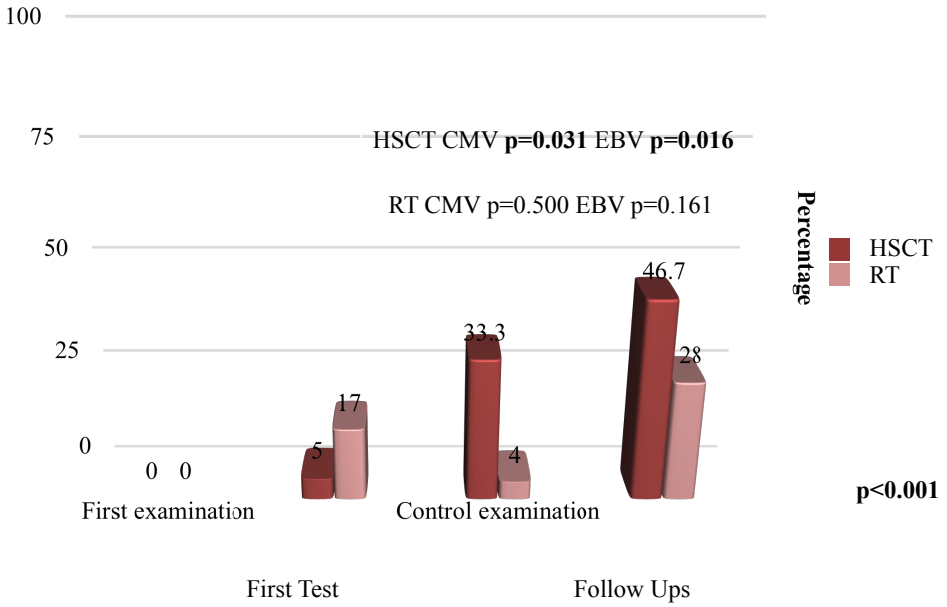


**Diagram 2.** Prevalence of positive CMV and EBV results among HSCT and RT recipients at the first test and follow ups during 2015.

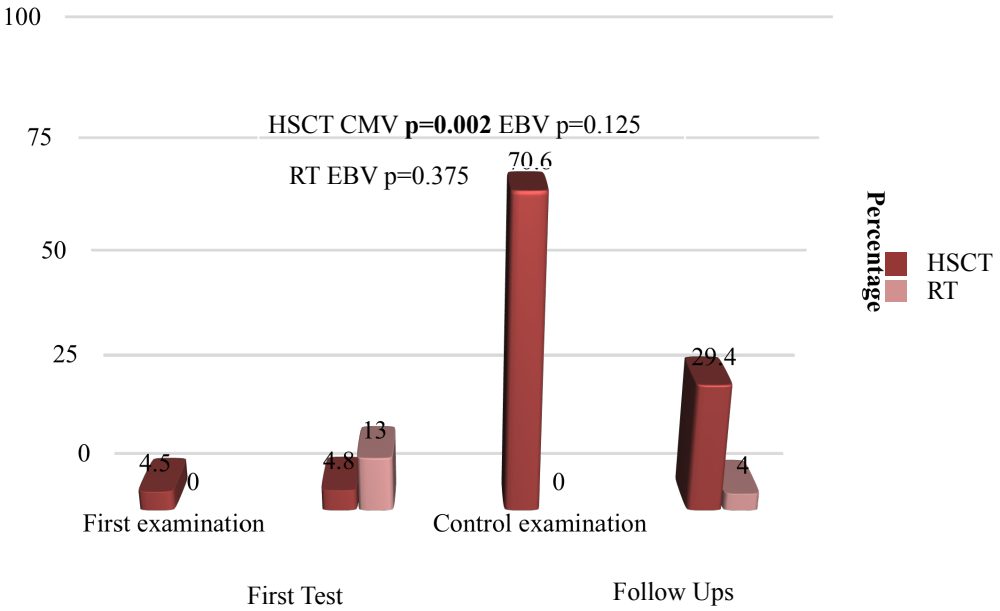


**Diagram 3.** Prevalence of positive CMV and EBV results among HSCT and RT recipients at the first test and follow ups during 2016.

## Discussion

The more potent immunosuppressive therapy successfully reduced the incidence of acute rejection and improved graft outcomes, however it also resulted in a higher incidence of viral complications (14). Solid organ transplant recipients, without preexisting immunity, are at the highest risk of developing diseases associated with herpesviruses, especially when they receive an organ from a seropositive donor (15). Human herpesviruses have a unique capability to establish a lifelong latent infection in the host and reactivation of viral infections is a common cause of hospitalization in transplanted patients (15,16). These infections are associated with certain adverse effects, which include an increased rate of graft rejection, opportunistic infections and malignancies (17). The goal of viral monitoring is the detection of subclinical viral infection, either preventing progression to viral disease or leading to early diagnosis of viral disease, which is associated with improved outcomes (14).

Statistically younger patients are among the HSCTR, making it unlikely they had contact with the herpesviruses at the age they were transplanted. On the other hand the RTR, which were statistically older than the HSCTR, had plenty of opportunities to come into contact with the herpesviruses ($p<0.001$). Similarly, Robertson JD discovered that in contrast to other solid organ transplant recipients, RTR are generally older, predominantly due to the availability of dialysis as a long-term overpass to transplant (18).

The significant impact of CMV infection and progression to CMV clinically apparent disease among HSCTR, has been reduced by prophylactic, preemptive, and beneficial treatments, using ganciclovir, valganciclovir, foscarnet, and cidofovir (19). Knowing the patient's serostatus before transplantation is important because the risk of occurrence of clinically manifested infections can be predicted (20).

According to the results of our research, HSCTR are in a greater risk of reactivation of CMV infection, comparing to RTR. Ozdemir et al. reached a comparable conclusion that 30% of HSCTR experienced late CMV reactivation, and 93% within a year from the transplantation (20).

The incidence of CMV in the RTR population is estimated to be between 8 and 32%, according to Cupic et al (21), and according to Smith JM, approximately 65% of pediatric RTR are CMV seronegative at the time of transplant (14). Likewise, RTR included in this study demonstrated a very low prevalence of positive CMV results, during the whole study period. Donor seropositivity, especially in the absence of prior recipient infection, is the most important risk factor for post - transplant infection, rendering the recipients at risk of invasive CMV disease, recurrent CMV, and ganciclovir - resistant CMV infection (21). Nowadays, routine monitoring allows for early detection of active CMV infection and the introduction of pre-emptive therapy with a goal to prevent overt CMV disease from occuring. The clinical value of quantitative plasma viral load measurements for prediction of CMV disease, was studied by Humar et al. They indicate the significance of pre and post-transplant screening protocols and report a modest value of regular CMV plasma viral load measurements in predicting CMV disease (23).

The greatest risk presents EBV infection, due to it's oncogenic potential and the primary goal is to prevent the development of post - transplant lymphoproliferative disorder (PTLD). The greatest risk for PTLD have the recipients which are EBV seronegative before transplantation and receive an organ from seropositive donor, therefore it is most commonly seen in pediatric and young adult populations. Post - transplant lymphoproliferative disorder can be present in many organs, including the allograft itself (21). According to Cukuranovic et al. RTR have the lowest risk of acquiring PTLD in comparison with other transplant populations, approximately 1-3% (22). A similar conclusion can be drawn from this research, considering the low prevalence of positive EBV results among the RTR.

Hematopoetic Stem Cell Transplants were monitored on all four expected herpesviruses, in contrary to RTR for which HSV1/2 and HHV6 were not expected and were rarelly tested for. However, this does not diminish the importance of these viruses in the post - transplant period, especially HHV6, which together with CMV and EBV, is a cofactor in the appearance of clinically manifested CMV or EBV infections or tumors. Perhaps, RTR are in a greater risk when infected by JC or BK viruses, due to their ability to establish latent infections within the kidneys and cause renal transplant dysfunction and/or loss of the graft, with manifestations like intestinal nephritis, ureteral stenosis or ureteral stricture (21).

## Conclusion

In consideration of these results, HSCTR are in a greater risk of CMV and EBV infections, compared to the RTR, due to their high prevalence of positive virology test results. Therefore, CMV and EBV post - transplant monitoring is important, especially in pediatric HSCTR, but it could also be useful for identifying other transplant recipients at high risk for symptomatic herpesvirus infection during the post - transplant period.

## References


1. L'Huillier AG, Kumar D. Immunizations in solid organ and hematopoetic stem cell transplant patients: A comprehensive review. Hum Vaccin Immunother. 2015;11:2852–63.
2. Hołowiecki J. Indications for hematopoietic stem cell transplantation. Pol Arch Med Wewn. 2008; 118:658-663.
3. Villarreal CDV, Perez JCJ, Alanis JCS, Candiani JO. Cutaneous graft-versus-host disease after hematopoietic stem cell transplant. An Bras Dermatol. 2016; 91:336–43.
4. Freeman MA, Myaskovsky L. An overview of disparities and interventions in pediatric kidney transplantation worldwide. Pediatr Nephrol. 2015; 30:1077–86.
5. Davis ID, Bunchman TE, Grimm PC, Benfield MR, Briscoe DM, Harmon WE, et al. Pediatric renal transplantation: indications and special considerations. Pediatr Transplant. 1998; 2:117–29.
6. Karbasi-Afshar R, Saburi A, Taheri S. Cardiovascular manifestations of allograft dysfunction in renal transplant recipients. Arab J Nephrol Transplant. 2014; 7:83–9.
7. Anastasopoulos NA, Duni A, Peschos A, Agnantis N. and Dounousi E. The spectrum of infectious diseases in kidney transplantation. In Vivo. 2015; 29:415-422.
8. Breuer S, Rauch M, Matthes-Martin S, Lion T. Molecular diagnosis and management of viral infections in hematopoietic stem cell transplant recipients. Mol Diagn Ther. 2012; 16:63–77.
9. Aiello FB, Calabrese F, Rigotti P, Furian L, Marino S, Cusinato R, et al. Acute rejection and graft survival in renal transplanted patients with viral diseases. Mod Pathol. 2004; 17:189–96.
10. Patrick R. Murray PhD, Ken S. Rosenthal PhD and Michael A. Pfaller MD, editors. Medical Microbiology, 7th edition. Elsevier Health Sciences; 2013.
11. Julio C. Mendez, Mark J. Espy, Thomas F. Smith, Jennie A. Wilson and Carlos V. Paya. Evaluation of PCR Primers for Early Diagnosis of Cytomegalovirus Infection following Liver Transplantation. J Cl Micro. 1998; 0095-1137
12. Li DJ, Bei JX, Mai SJ, Xu JF, Chen LZ, Zhang RH, et al. The dominance of China in the spectrum of Epstein-Barr virus strains from Cantonese patients with nasopharyngeal carcinoma. J Med Virol. 2009; 81:1253-1260
13. Daibata M, Taguchi T Nemoto Y, Taguchi H, Miyoshi I. Inheritance of Chromosomally Integrated Human Herpesvirus 6 DNA. Blood 1999; 94:1545-9
14. Smith JM, Dharnidharka VR. Viral surveillance and subclinical viral infection in pediatric kidney transplantation. Pediatr Nephrol. 2015; 30:741–748.
15. Smith C, Khanna R. Immune regulation of human herpesviruses and its implications for human transplantation. Am J Transplant. 2013; 13:9–23.
16. Sandherr M, Hentrich M, von Lilienfeld-Toal M, Massenkeil G, Neumann S, Penack O, et al. Antiviral prophylaxis in patients with solid tumours and haematological malignancies. Ann Hematol. 2015; 94:1441–50.
17. Fishman JA. Overview: Cytomegalovirus and the herpesviruses in transplantation. Am J Transplant. 2013; 13:1–8.
18. Robertson J.D. Pediatric transplantation: preventing thrombosis. J of Thromb and Haem. 2015; 1:S351-61.
19. Campos AB, Ribeiro J, Boutolleau D,Sousa H. Human cytomegalovirus antiviral drug resistance in hematopoietic stem cell transplantation: current state of the art. Rev Med Virol. 2016; 26:161-82.
20. Ozdemir E, Saliba RM, Champlin RE, et al. Risk factors associated with late cytomegalovirus reactivation after allogeneic stem cell transplantation for hematological malignancies. Bone Marrow Transplant. 2007; 40:125-36.
21. Cupic M, Lazarevic I, Pravica V, Banko A, Karalic D, Naumovic R. et al. The Prevalence of the Most Important Viral Infections in Renal Transplant Recipients in Serbia. Arch Biol Sci Belgrade. 2012; 64: 1285-1296.
22. Cukuranovic J, Ugrenovic S, Jovanovic I, Visnjic M, Stefanovic V. Viral infection in renal transplant recipients. Sci World J. 2012; 2012:820621
23. Humar A, Paya C, Pescovitz MD, Dominguez E, Washburn K, Blumberg E, et al.Clinical utility of cytomegalovirus viral load testing for predicting CMV disease in D+/R- solid organ transplant recipients. Am J Transplant. 2004;4:644-9.
24. E, Magkos F, Schulpis KH et al.The effect of MTHFR (C677T) genotype on plasma homocysteine concentrations in healthy children is influenced by gender. Eur J Clin Nutr. 2006; 60(2):155-162.