Original article

Cytomegalovirus Infection in Kidney Transplant Recipients

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Abstract

Introduction: Present study examined the frequency of CMV infection during follow-up using quantitative nucleic acid amplification testing, the frequency of administration of infection prophylaxis, viremia and infection in kidney transplant recipients who underwent transplantation (TX) at the University Hospital Center Osijek.

Materials and Methods: 107 kidney recipients who underwent transplantation in the period 20 October 2007 – 24 August 2016 were included. Demographic and clinical data, data about pre-transplantation CMV IgG test results of recipients and their donors, data about CMV prophylaxis, viremia, infection, and kidney transplant function were taken from medical records and analyzed.

Results: 92.5% of kidney recipients and 86% of donors were CMV IgG positive before TX. 28% of recipients were CMV-DNA positive at some point after TX, none of whom received a transplant from an IgG negative donor. 89.7% of participants received CMV prophylaxis. Seven participants developed CMV disease, 2 of whom were not administered prophylaxis. Participants were tested for CMV-DNA once a year (median; min 0 max 6). CMV disease was marginally more frequent in those who did not receive valganciclovir prophylaxis (P = 0.066).

Conclusion: It seems wise to enforce the administration of CMV prophylaxis and CMV-DNA testing in accordance with protocol, in order to detect viremia on time and to implement preemptive treatment, aiming at prevention of clinical manifestation of infection and preservation of graft function.

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Introduction

Cytomegalovirus (CMV) infection is one of the most common opportunistic infections in kidney transplant recipients. There are two ways of acquiring the infection: reactivation of the virus or de novo infection of an immunocompromised patient. The infection can be asymptomatic, it can jeopardize the function of the graft and/or cause a systemic infection and even death.

CMV infection is defined as virus isolation or detection of viral proteins (antigens) or nucleic acid in any bodily fluid or tissue specimen, regardless of symptoms. CMV disease is defined as evidence of CMV infection with attributable symptoms and can be further categorized as a viral syndrome (i.e. fever, malaise, leukopenia, and/or thrombocytopenia), or as a tissue-invasive ("end organ") disease.

In recent years, administration of CMV infection prophylaxis during the first post-transplantation months has become a standard part of therapy after kidney transplantation (TX). It is also recommended to observe CMV viremia using quantitative nucleic acid amplification testing (QNAT) that detects CMV-DNA (deoxyribonucleic acid). Prophylaxis and diagnostics raise the cost of treatment. However, modern protocols justify this approach with the lower total cost of the treatment, since it is deemed that this approach leads to fewer complications.

The prophylaxis and diagnostics protocol at the University Hospital Center (UHC) Osijek is not strict and has changed in recent years, while the approach to prophylaxis and diagnostics is not completely uniform. The aim of the study was to examine the frequency of CMV infection during follow-up using quantitative nucleic acid amplification testing (QNAT) for CMV-DNA, the frequency of administration of CMV infection prophylaxis, and the frequency of CMV viremia and infection in kidney transplant recipients who received their transplant in UHC Osijek during the period between 20 October 2007 and 24 August 2016.

Materials and Methods

The study included 107 participants, 60 men and 47 women, who underwent kidney TX in UHC Osijek in the period between 20 October 2007 and 24 August 2016. Median age at the time of the TX was 51 (min. 27, max. 71), while at the time of the study it was 57 (min. 32, max. 74). Research methods included collecting data from medical records and statistical analysis. The following data were analyzed: demographic data of the participants (age, sex), clinical features of the participants (primary kidney disease, data about dialysis and TX), data about CMV IgG test results of both recipients and their donors prior to TX, data about CMV prophylaxis, viremia, infection, and about kidney transplant function.

Statistical analysis

Data analysis was conducted using SPSS for Windows (version 16.0, SPSS Inc., Chicago, IL, USA). Nominal data were expressed as absolute and relative frequencies. Numerical data were expressed as mean and standard deviation (SD) in case of normal distribution, and as median and range (min. – max. or interquartile range, IQR) in case of asymmetric distribution. The Kolmogorov-Smirnov test was used to analyze the normality of distribution of the variables. Differences in frequencies were tested using the chi-square test, in numeric variables of normal distribution using Student's t-test and Mann-Whitney U test by asymmetric distribution. Correlations between numeric variables were tested using Spearman's Rho test. Statistical significance was accepted at $P < 0.05$.

Results

For 97 kidney recipients (90.7%) this was the first TX, for 7 of them (6.5%) the second, and for 3 of them (2.8%) the third one. Table 1 shows the demographic data of the participants. Female participants were significantly older both at the time of the study (Mann-Whitney U test, $P = 0.003$) and at the time of the TX (Mann-Whitney U test, $P = 0.001$). By September 2016, 12 (11.2%) participants, 6 men and 6 women, had died, one of whom was CMV IgG negative before the TX.

There was no significant difference in mortality between men and women (Mann-Whitney U test, P = 0.654).

Table 1. Demographic data of patients

Number of patients (%)		**Male** 60 (56%)	**Female** 47 (44%)
Age (median in years)	At the time of the study (IQR)	53 (45–59)	60 (53–65)
	At the time of the TX* (IQR)	50 (42–54)	55 (49–61)

*transplantation

Table 2. CMV* serology data

			N	**%**
CMV IgG prior to TX	Recipient	Positive	99	92.5
		Negative	8	7.5
	Donor**	Positive	92	86
		Negative	14	13.1
Recipient–donor pairs based on CMV serology		R+/D+	84	79.2
		R+/D-	14	13.3
		R-/D+	8	7.5
		R-/D-	0	0

*cytomegalovirus, **data for one donor were unavailable

R – recipient, D – donor

Table 2 shows the CMV serology data. Protocolar administration of CMV prophylaxis was introduced in February 2009. Table 3 shows data related to prophylaxis administration and development of CMV infection and disease. Two out of 3 (66.7%) participants who were not administered prophylaxis and 5 out of 27 (18.5%) of those who were administered prophylaxis developed a clinical manifestation of disease, with no significant (rather, marginal) difference in occurrence (Mann-Whitney U test, P = 0.066). Sixteen (53.4%) positive participants were men, while 14 (46.6%) were women. No significant difference was found in the frequency of CMV-DNA positivity between the sexes (Mann-Whitney U test, P = 0.671).

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Table 3. CMV* prophylaxis and infection data

			N	%
Prophylaxis	Administered		96	89.7
	Not administered		11	10.3
	Duration (mean, in months + SD)		4 ± 2	
CMV infection and disease	Positive for CMV-DNA**	Total	30	28
		Received prophylaxis	27	90
		Did not receive prophylaxis	3	10
		CMV IgG*** positive prior to TX	26	86.7
		CMV IgG negative prior to TX	4	13.3
		R+/D+	26	86.7
		R-/D+	4	13.3
	CMV disease	Total	7	10
		Received prophylaxis	5	71.4
		Did not receive prophylaxis	2	28.6

*cytomegalovirus, **positive for CMV-DNA was defined as having > 1000 copies/mL, ***immunoglobulin G, R – recipient, D – donor

The group in which the recipient was CMV IgG positive and the donor negative was significantly less often CMV-DNA positive at some point after the TX in comparison with the group in which the recipient was CMV IgG negative and the donor positive (Mann-Whitney U test, P = 0.002), and in comparison with the group in which both were CMV IgG positive (Mann-Whitney U test, P = 0.016). There was no significant difference in the frequency of positivity at some point after the TX between the group in which both were positive and the one in which the recipient was CMV IgG negative and the donor positive (Mann-Whitney U test, P = 0.159). No recipient of a CMV IgG negative donor became CMV-DNA positive. There was no pairing in which both the recipient and the donor were CMV IgG negative.

Table 4 shows data related to CMV-DNA testing and follow-up. According to the kidney function criteria for prophylaxis dosing, the participants received the appropriate drug dose. Table 5 shows graft function data. There was no significant difference between the sexes in creatinine concentration in the serum at the start of administration of prophylaxis (Mann-Whitney U test, P = 0.365). The group in which the recipient was CMV IgG positive and the donor negative had significantly lower serum creatinine concentration at the end of prophylaxis administration compared to the group in which both were CMV IgG positive (Mann-Whitney U test, P = 0.009). Women had significantly lower serum creatinine concentration than men at the end of prophylaxis (Mann-Whitney U test, P = 0.002). Seven out of 30 participants (23.3%) who were CMV-DNA positive at some point after the TX developed CMV disease. In 2 of them, it manifested as invasive CMV disease, while in the other 5 it manifested as a viral syndrome with leukopenia, fever, malaise, loss of appetite, diarrhea, and weight loss.

Table 4. CMV-DNA* testing and follow-up data

		N
Number of tests performed	Total	442
	Per patient (median + IQR)	4 (2–6)
	Per patient year	1.03
	Patient years	426
Follow up duration	median + IQR	4 (2–6)
	Min – max	0–9

*cytomegalovirus deoxyribonucleic acid

Table 5. Graft function data

			Value	
	Start of prophylaxis – median (IQR)		35 (19.5–48.5)	
			N	**%**
GFR* (in mL/min/1.73 m²)	Value	> 60	14	14.6
		40–59	26	27.1
		25–39	23	24
		10–24	24	25
		< 10	6	6.3
		Unavailable	3	3.1
				Median (IQR)
Creatinine (µmol/L)	Start of prophylaxis	Men		187 (127–264)
		Women		152 (119–262)
	End of prophylaxis	Men		129 (111–145)
		Women		103 (93–141)
		R+/D+		128 (101–148)
		R+/D-		106 (94–110)
		R-/D+		119 (99–166)

*glomerular filtration rate, R – recipient, D – donor

Discussion

Kidney TX has been performed at the UHC Osijek since 2007, and the availability of CMV-DNA diagnostics dates back to 2009. Before 2007, patients treated at the UHC Osijek dialysis department had their TX at UHC Zagreb, UHC Rijeka or Merkur Clinical Hospital, and CMV diagnostics was performed in Zagreb or Rijeka. This study included only the patients whose TX and diagnostics were performed in Osijek.

CMV is widely present in the population, which has been shown in numerous studies, according to which the prevalence of seropositivity to CMV IgG ranges from 30 to 97% (1, 2). Results of this study agree with those results, finding that 92.5% of recipients and 86% of their donors were CMV IgG positive prior to the TX. This could be considered as a high prevalence.

Since CMV presents a significant risk of morbidity and mortality in the population of persons who have received transplants, the importance of prevention of reactivation or de novo infection has been recognized, whether it is performed by administering prophylaxis or through preemptive treatment (3 – 7, 14). Prevention of CMV infection at the UHC Osijek started in February 2009 with administration of oral valganciclovir, at first during 3 months for CMV IgG positive and 6 months for CMV IgG negative recipients, and since October 2014, a universal 6-month prophylaxis has been in use.

Taking that into consideration, 96 of 107 kidney recipients, or 89.7%, were administered prophylaxis, which is a large share of recipients.

The standard dose of oral valganciclovir in CMV prophylaxis is 900 mg per day. That dose is adjusted for renal function, an indicator of which is creatinine clearance (or calculated GFR). Dose adjustment is extremely important since valganciclovir can cause nephrotoxicity and thus jeopardize the graft function. Some of the side effects of valganciclovir, such as leukopenia, nausea, diarrhea, and elevated serum liver enzymes, overlap with the symptoms of CMV infection and disease, which is why finding the actual cause of such symptoms is of great importance.

Regular testing for CMV-DNA plays an important role, and it can help with early detection of infection and administration of preemptive treatment already at low viral loads, which could lead to prevention of clinical manifestations and better preservation of graft function, since preemptive treatment has been linked to fewer toxic effects on the transplanted kidney in comparison with the prophylaxis (8). CMV infection and disease can, however, appear despite preventive therapy, and the reasons for this are incorrect dosing, discontinuation of preventive therapy, or simply a failure of such therapy (4).

Thirty recipients, or 28% of those who were included in the study, developed CMV infection (and 7 of them had CMV disease), which is a relatively high number if we consider the fact that most of them took prophylaxis. A primary risk factor for the development of CMV viremia and disease is considered to be the serostatus of the recipient–donor pair regarding CMV IgG antibodies; the pairs in which both are positive and in which the recipient is negative and the donor positive are under increased risk for the development of CMV disease (9 – 12). Most of the CMV-DNA positive recipients in this study come from a recipient–donor pair in which both were CMV IgG positive, 26 out of 30 in total, which is 86.7% of all positive recipients. The other 4 CMV-DNA positive participants come from a recipient–donor pair in which the recipient was CMV-DNA negative and the donor positive.

Considering the fact that recipient–donor pairs in which both were CMV IgG positive far outnumbered participants with other serological combinations, it is necessary to mention the share of CMV-DNA positive participants in each group. There were 84 participants belonging to a pair in which both the recipient and the donor were CMV IgG positive, and out of them all, 26 were CMV-DNA positive at some point after the TX, which constitutes 31% of such participants. Seven participants belonged to a recipient–donor pair in which the recipient was CMV IgG negative and the donor positive. Among them, 4 participants became CMV-DNA positive. The remaining 14 participants for whom the serostatus combination is known belonged to a recipient–donor pair in which the recipient was CMV IgG positive and the donor was negative; none of them became CMV-DNA positive. There were no recipient–donor pairs in which both were CMV IgG negative. These findings coincide with previous studies from the pre-prophylaxis era, which studied the natural course of CMV infection in kidney transplant recipients and showed that 56% of kidney transplant recipients from a recipient–donor pair in which the recipient is CMV IgG negative and the donor positive develop CMV disease after TX, as do 20% of those from a pair in which both the recipient and the donor are CMV IgG positive (12).

The participants of this study who received a kidney transplant from a CMV IgG negative donor (all of them were CMV IgG positive in this study), in addition to never becoming CMV-DNA positive after the TX, also had better kidney transplant function compared to those who received their graft from a CMV IgG positive donor. Lack of viremia in such participants was accompanied by better kidney transplant function. There was no significant difference in the occurrence of CMV-DNA positivity between the participants who took prophylaxis and those who did not, but CMV disease was more common in those who did not take it, with marginal significance (P = 0.066). However, with an insufficiently large sample of participants who did not take prophylaxis, the rarity of clinical

manifestations of infections in relatively common viremia could still be interpreted as a consequence of administered prophylaxis and timely detection of viremia with regular testing for CMV-DNA.

Out of 7 participants who had clinical symptoms, 2 developed CMV disease and 5 of them had CMV syndrome. One participant from the pre-prophylaxis era developed early invasive CMV disease and died as a result. One participant developed CMV disease, which manifested in elevated serum liver enzymes and esophagitis. Of the participants who had CMV syndrome, 1 participant had elevated serum liver enzymes, 1 had leukopenia and 1 had weight loss. One participant had diarrhea, fever, and malaise, and 1 had diarrhea and loss of appetite. All of them, except for the deceased one, were successfully treated with antiviral medication.

Previous studies have shown that gastrointestinal symptoms were the most common manifestations of CMV infection and disease (3, 13), which coincides with our findings. Participants were tested for CMV-DNA once a year on average, but the frequency of such testing greatly varied from participant to participant, with some of them not being tested for years during certain periods. Such findings, together with the relatively frequent CMV viremia in the observed population, and the significance which CMV infection has for graft function and overall survival of kidney transplant recipients, lead us to conclude that more work is needed to develop a standardized protocol for prevention of CMV infection in kidney transplant recipients.

The limitations of this study, in the sense of evidence-based medicine, were the epidemiological character (design) and the absence of comparable data in a scenario without prophylaxis, since diagnostics, screening and follow-up of CMV were rare and deficient in the pre-prophylaxis era. Other than that, there remains an important aspect of this problem, which could complement this study in the future. It is the immunosuppressive protocol that was part of the participants' treatment, its dynamics over time, with respect to both the year of TX and the protocols in force during that time, as well as complications and comorbidities other than CMV. Likewise, the promptness of the valganciclovir dose adjustment in relation to kidney function dynamics should be studied, although it appears adequately adjusted at the two studied points of time – at the beginning and at the end of administration of prophylaxis.

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Competing interests. None to declare.

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