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Original article

MONITORING OF CMV INFECTION IN KIDNEY TRANSPLANT RECIPIENTS

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ABSTRACT

Human cytomegalovirus is a ubiquitous herpesvirus that establishes lifelong latency after primary infection, but can cause life-threatening disease in immunosuppressed patients. CMV invasive disease leads to significant morbidity and mortality following kidney transplantation.

We tested 2 groups of patients - Group A included 20 potential kidney recipients and 29 potential donors investigated by ELISA and Group B included 53 adult kidney transplant recipients all of them tested in ELISA and 24 of them tested in QRT-PCR for CMV-DNA from plasma samples.

In group A 16 (80%) of 20 potential kidney recipients were anti-CMV IgG positive and 4 (20%) were anti-CMV IgG negative. Twenty eight of 29 potential donors were found seropositive, and only one was not infected.

In group B overall 119 ELISA tests for specific anti-CMV antibodies were performed. Anti-CMV IgM negative was 68 (57%) of the tested samples, twelve (10%) showed anti-CMV IgM equivocal results and 39 samples (33%) were with anti-CMV IgM positive. Seven of them (13,2%) showed repeatedly anti CMV IgM positive results. All 119 (100%) displayed ànti-CMV IgG positive results.

Overall 41 PCR analyses from plasma samples of 24 kidney transplant recipients (group B) were performed. CMV-DNA replication was detected in 5 plasma samples obtained from 3 patients (12.5%) at a different time - from 20 days till almost 8 years after the transplantation.

Despite the high seroprevalence to CMV 20% of the potential recipients were at high risk of primary infection when receiving a kidney from a seropositive donor.

Positive serological results during the regular post-transplantation monitoring complemented with or without clinical data are indicative and require further QRT-PCR analysis.

Keywords: CMV, kidney transplant recipient (KTR), seroepidemiologic screening, ELISA, QRT-PCR studies

INTRODUCTION:

Human cytomegalovirus (CMV) is discovered in the 1950s and is one of the largest known humans enveloped DNA â-herpesviruses [1]. It is ubiquitous and like other members of the herpesvirus family establishes lifelong latency after primary infection [2]. Usually, the first exposure to CMV occurs during childhood, and it is widely distributed in 30% to 97% of the general population depending on the age and socio-economic status [3]. In immunocompetent individuals, CMV infection is a benign, sub-clinical, with the lack of detectable virus, a hallmark of latent infection. In contrast, reactivation of latency in immunocompromised individuals can result in life-threatening or end-organ disease [4]. CMV primary infection, reinfection or reactivation is one of the most frequent opportunistic infections following kidney transplantation and may be a risk for acute rejection in renal transplanted recipients [5]. CMV invasive disease leads to significant morbidity and mortality as well, following kidney transplantation. The development of severe invasive forms is uncommon nowadays with the post-transplantation monitoring, prophylactic regimens in high-risk patients and early pre-emptive treatment with ganciclovir [6]. In order to reduce the impact of CMV on transplant outcomes, there have been made remarkable efforts to improve its diagnosis, prevention, and treatment. Despite these significant advances, CMV continues to have a major impact on patients and allograft survival among kidney transplanted recipients through a variety of direct and indirect effects [7].

Aims:

1) To estimate the frequency of CMV infection in patients candidates for kidney transplantation in ''St. Marina'' University Hospital, Varna, Bulgaria and to determine the risk groups for primary CMV infection and CMV reactivation/reinfection.

2) To monitor the infection in patients following kidney transplantation with both serological (ELISA) method and Quantitative Real time amplification technique (QRT PCR) for CMV-DNA detection.

MATERIALS AND METHODS:

Study population:

Group A included 20 potential kidney recipients (11 males and 9 females) and 29 potential kidney donors with

preliminary serological screening for CMV infection.

Group B included 53 adult kidney transplant recipients (KTR) (26 males and 27 females). They were monitored for CMV infection by ELISA in different intervals after transplantation preventively or on demand when clinical evidence of reactivation was detected, or when clinical signs of allograft dysfunction occurred as follows: 10 patients were repeatedly tested in certain intervals (1 week, 1 month, 3 months, 6 months, subsequent years and on demand), 25 patients were tested twice, and 18 patients were tested only once. One of the patients rejected the allograft and one died.

For 24 of all 53 kidney transplant patients were performed tests both by QRT-PCR and ELISA for a period of one and a half years. A total of 41 EDTA blood samples were obtained from them. Nine of these patients were retested at certain intervals after transplantation and/or on demand when clinical evidence of reactivation was detected. Fifteen of the patients were tested only once.

Methods:

1. Commercial ELISA test kits for detection of specific anti-CMV-IgM and IgG (EUROIMMUN, Germany) were performed according to the producer's recommendations.

2.PCR analysis:

DNA extraction was performed with DNA/RNA Prep, nucleic acid extraction kit for the extraction and purification of total RNA/DNA (Sacace, Italy, Biotechnologies) from 150 µl plasma in final volume 50 µl, according to the producer's recommendations.

DNA amplification was carried out using commercial Taq-man Quantitative Real Time

PCR (QRT-PCR) for CMV-DNA detection (Sacace, Italy, Biotechnologies), according to the producer's recommendations with PCR instrument Quant Studio Dx in 25 µl reactions.

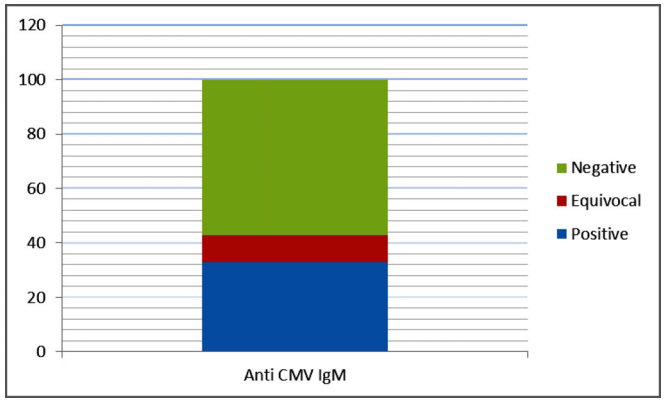
RESULTS:

In group A preliminary serological CMV screening results indicated that 16 (80%, 95% CI: 62.5% to 97.5%) of the potential recipients were anti-CMV IgG positive and 4 (20%, 95% CI: 2.5% to 37.5%) were anti-CMV IgG negative. Twenty eight of 29 potential donors were found seropositive, and only one was not infected.

In group B overall 119 ELISA tests for specific anti-CMV antibodies were performed.

Sixty eight (57%, 95% CI: 48.1% to 65.9%) of the samples indicated anti-CMV IgM negative results, 12 (10%, 95% CI: 4.61% to 15.4%) - anti-CMV IgM equivocal results and 39 (33%, 95% CI: 24.6% to 41.5%) - anti-CMV IgM positive results. Seven of them (13,2%) showed repeatedly anti CMV IgM positive results. All 119 (100%) serum samples show anti-CMV IgG positive results (Fig.1).

Fig.1. Serological monitoring of investigated 53 KTR (group B)



Of the 53 kidney transplanted recipients (Group B) 10 were repeatedly tested, and 7 of them (13,2%) showed periodically anti-CMV IgM positivity at certain intervals after the transplantation (Table 1).

The serology tests results revealed reactivation of latent CMV infection by elevation of anti-CMV IgM titers or de novo positivity of IgM antibodies, after previous negative results. The rest 3 patients were anti-CMV IgM negative in every serology testing after the transplantation (Table 1).

Table 1. Serological monitoring of 10 KTR according to the different periods after the transplantation.

Patient	Time after kidney transplantation									
	1 w.	1 m.	3 m.	6 m.	1 y.	2 y.	3 y.	4 y.	6 y.	7 y.
ILA	IgM-		IgM+	IgM+	IgM-			IgM-	IgM+	IgM-
KST		IgM-		IgM+	IgM+	IgM-	IgM-		IgM-	
NSN	IgM-		IgM+	IgM+	IgM+	IgM+	IgM+	IgM+	IgM+	IgM+
ATM	IgM-			IgM+	IgM-					
AMH				IgM+	IgM+	IgM+	IgM+			
NAV			IgM+	IgM+	IgM+	IgM+	IgM+			
LSH			IgM+	IgM+	IgM+	IgM+	IgM+			
DFM				IgM-	IgM-	IgM-		IgM-		

SPP			IgM-	IgM-	IgM-	IgM-				
IIA			IgM-	IgM-	IgM-	IgM-				

For the purpose of modern CMV diagnostics, we have introduced the QRT-PCR analysis at a certain point. Overall 41 plasma samples from 24 KTR were tested both by QRT-PCR for CMV-DNA detection and by ELISA for antibody detection. Nine of the patients were retested at certain intervals after transplantation and/or on demand when clinical evidence of reactivation was detected. Fifteen of the patients were tested only once.

CMV-DNA replication was determined in 5 of 41 (12.2%, 95% CI: 2.2% to 22.2%) plasma samples obtained from 3 patients at a different time - from 20 days till almost 8 years after the transplantation (Table 2). The other 36 obtained plasma samples (87.8%, 95% CI: 77.8% to 97.8) had undetectable viral load (Table 3).

In ELISA analysis the 41 tested plasma samples showed overall 21 (51.2%, 95% CI: 35.9% to 66.5%) anti-CMV IgM negative results, 2 (4.9%, 95% CI: -1.7% to 11.5%) - equivocal results and 18 (43.9%, 95% CI: 28.7% to 59.1%) - anti-CMV IgM positive results. All 41 ELISA tests were anti CMV IgG positive (Table 3).

Table 2. A summary of KTR with CMV positive viral load.

Gender	Age	Time after kidney transplantation	QRT PCR for CMV	CMV load	Serology	Related symptoms
Female	34	20 days	Positive	6000 IU/ml	CMV IgM-/ CMV IgG +	Fever
Male	31	3 years	Positive	123 IU/ml	CMV IgM+/ CMV IgG +	Fever, elevated creati nine levels and urinary tract infection
Female	32	7 years	Positive	538 IU/ml	CMV IgM+/ CMV IgG +	Fever, malaise
	33	7,5 years	Positive	832 IU/ml	CMV IgM+/ CMV IgG +	Malaise, Repeating herpes labialis
	33	7,5 years	Positive	1370 IU/ml	CMV IgM+/ CMV IgG +	Fever, urinary tract infection

Table 3. Comparative results for 41 plasma samples in ELISA and QRT PCR in 24 KTR (group B)

Anti-CMV IgM			Anti-CMV IgG Positive	CMV-PCR	CMV-PCR
Negative (-)	Equivocal (+/-)	Positive (+)	(+)	(-)	(+)
21/41	2/41	18/41	41/41	36/41	5/41
(51.2%)	(4.9%)	(43.9%)	(100%)	(87.8%)	(12.2%)

DISCUSSION:

According to our previous data, CMV infection is widespread in Bulgaria with total seroprevalence 78.4% in the Northeastern population [8]. Such high seroprevalence may have a profound effect on the CMV morbidity and CMV invasive disease following transplantation. Laboratory diagnosis of CMV disease is considered in cases of malaise, fever, leucopenia and end-organ affection are found, and no other obvious cause could be identified and if there is a clinical response to a reduction in immunosuppression or ganciclovir therapy [9].

CMV disease risk is highest when primary infection occurs in seronegative transplanted recipients with the transplanted organ from the seropositive donor (D+R-) [10]. In our survey of the preliminary serologically screened recipients and their donors, only 4 (20%) of the recipients were at hgh risk of primary infection after transplantation when receiving a kidney from a seropositive donor. Other authors report 23% of the renal allograft recipients belonged to the high-risk CMV mismatch group [11]. In our study, one of the patients identified with high risk was found with high viral load (6000 IU/ml) 20 days after the transplantation probably because of primary infection with the transplanted organ. The other 3 high risk patients became seropositive after the transplantation probably because of infection from the donor organ. We have not detected CMV viral load in their plasma samples later. The rest 16 potential recipients were not at high risk of developing primary CMV infection and invasive CMV disease because of the pre-existing immunity. Reinfection or superinfection imported with the transplanted organ is more likely to occur in their cases. According to the literature, such condition occurs between 30% and 80% of patients undergoing solid organ transplantation, although its incidence and the presence of symptoms vary

depending on the type of transplant, the presence of risk factors and prophylactic strategies [12]. In another study, carried out in China center for transplantation the total incidence of CMV reactivation in 319 kidney transplanted patients was determined with QRT PCR as 8.8%. To the authors, the patients within 3 to 6 months and 5 to 10 years after transplantation had a higher risk of CMV infection [13]. The number of our patients tested is small, but our positive QRT PCR results are in the same periods after the transplantation and with similar frequency (Table 3).

Serological methods for determining antibody responses to CMV have commonly been used in our country. But a key diagnostic issue for CMV infection is distinguishing between active disease and latent infection [7]. Before transplantation, serological CMV markers are reasonable as a base line and can be used in order to adjust for the risk of CMV disease [14]. However, after transplantation, they have only limited value. Studies conducted by Flechner et al. [15] and Prieser et al. [16] have reported that serological assays lack the usefulness in diagnosing CMV reactivation in transplant patients. In some cases, antibodies may not develop due to immunosuppression, or develop after the disease is already cured, or may persist for years. Similar findings have been observed in our study. The total incidence of CMV reactivation determined with QRT PCR was found 12.5% (n=3/24). Serological data for reactivation (anti-CMV IgM positive) we found in 13.2% of the repeatedly tested patients (n=7/53). One of the positive in QRT PCR patient with high viral load was anti-CMV IgM negative, probably due to immunosuppression after the transplantation and IgM titres may not be raised to the detectable level. Positive serological results during the regular monitoring, complemented with or without clinical data are indicative and require further QRT PCR analysis. We continue our work and collaboration with clinicians in the field of CMV infection monitoring in kidney transplant recipients to prevent serious, life-threatening reactivations.

CONCLUSIONS:

1. Despite the advances in the prevention and management of CMV infection in kidney recipients it continues to have a significant impact on this risk group.

2. Immunological CMV status before transplantation should be performed for both recipients and donors, as in our patients.

3. The CMV negative recipients who received a kidney from CMV positive donors are with high-risk of developing of CMV disease and should be monitored with attention if that mismatch (D+/R-) could not be avoided.

4. The CMV positive recipients who receive a kidney from CMV positive donors should be tested for CMV reinfection/reactivation with both methods (ELISA and QRT PCR) in certain intervals in the early and the late post-transplant period for determination of appropriate treatment.

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