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CMV seropositivity does not increase the risk of death among elderly nursing home residents

Obecność przeciwciał przeciwko CMV w surowicy nie zwiększa ryzyka zgonu u osób w wieku podeszłym przebywających w domu opieki

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STRESZCZENIE

OBECNOŚĆ PRZECIWCIAŁ PRZECIWKO CMV W SUROWICY NIE ZWIĘKSZA RYZYKA ZGONU U OSÓB W WIEKU PODESZŁYM PRZEBYWAJĄCYCH W DOMU OPIEKI

Cel pracy. Celem pracy była ocena miana przeciwciał przeciwko wirusowi cytomegalii (CMV) w surowicy i obecności materiału genetycznego wirusa w osoczu u osób w wieku podeszłym przebywających w domu opieki oraz wpływu tych czynników na ryzyko zgonów tych osób.

Materiał i metodyka. Do badania włączono 202 rezydentów domu opieki w wieku 65 lat i powyżej. W czasie 3 letniej obserwacji, w latach 2015-2018, zmarło 126 rezydentów (62,4%). Materiał genetyczny wirusa w osoczu oznaczano przy pomocy metody łańcuchowej reakcji polimerazy z analizą w czasie rzeczywistym. Miano przeciwciał w surowicy oznaczano za pomocą komercyjnych testów ARCHITECT CMV test.

Wyniki. Nie stwierdzono obecności materiału genetycznego wirusa w osoczu. Nie stwierdzono różnicy w wartości miana przeciwciał przeciwko CMV w grupie osób, które przeżyły w porównaniu do zmarłych. Testem Chi-kwadrat Pearsona ani testem Fishera nie wykazano różnicy pomiędzy liczbą zgonów w grupie osób seronegatywnych, seropozytywnych < 250 IgG [Au/ml] i seropozytywnych > 250 IgG [Au/ml]. Wyniki te potwierdzono krzywymi przeżycia Kaplana-Meyera.

Wnioski. Wyniki badania wskazują, że zakażenie CMV oraz wartość miana przeciwciał anty CMV nie zwiększają ryzyka zgonów osób w wieku podeszłym.

Słowa kluczowe: **wirus cytomegalii, wiek podeszły, ryzyko zgonu**

ABSTRACT

CMV SEROPOSITIVITY DOES NOT INCREASE THE RISK OF DEATH AMONG ELDERLY NURSING HOME RESIDENTS

Aim. The aim of this study was to assess the anti-CMV antibody titre, the presence of genetic material of the virus in the plasma of elderly residents of nursing homes and the impact of the CMV infection on the risk of death.

Material and methods. The number of 202 residents of a nursing home in Warsaw, aged 65 and over, were observed for 1095 days (3 years) between 2015 and 2018. During this period 126 (62.4%) residents died. Plasma CMV DNA levels were assessed using real-time PCR. Anti-CMV antibody titre was measured with the use of commercially available ARCHITECT CMV test.

Results. No genetic material of the CMV was found in the studied group of the residents. The mean IgG titre did not differ between those who survived and those who deceased (p=1). Pearson's Chi-squared test and Fisher's exact test did not reveal any differences in the rate of deaths among the groups of seronegative, seropositive < 250 IgG [Au/ml], and seropositive > 250 IgG [Au/ml] residents. Kaplan-Meyers survival curves confirmed these results.

Conclusions. We did not demonstrate that CMV infection or the anti-CMV antibody titer have any effect on the risk of death in the study group.

Key words: **cytomegalovirus, elderly, mortality**

INTRODUCTION

High prevalence rates of Human cytomegalovirus (CMV) infection are observed worldwide, varying between 40 and 100%, depending on various socio-demographic factors, e.g. level of education, socioeconomic status, gender and age [1-2]. Persons of lower socioeconomic status are more likely to be CMV seropositive [3].

CMV seroprevalence was found to be the highest in South America, Africa and Asia and the lowest in Western Europe and the United States. Females generally have higher seroprevalence than males, although in most studies the differences were small [3]. Seroprevalence rates of the virus, typically acquired in childhood, increase steadily with age.

Seropositivity to and reactivation of CMV may play a key role in long-term health outcomes [4]. Some studies suggest that reactivation may be more frequent in healthy older individuals than in the young ones [5].

Epidemiological reports indicate a connection between CMV and the impairment of cognitive functions [6], weakness [7] and geriatric syndrome, which has poor prognosis that is independent of other aspects [8]. The virus has been implicated in the pathophysiology of numerous chronic diseases that include cardiovascular disease, depression and cancer [9, 10]. However, there is controversy in data regarding the association of serostatus of residents and anti-CMV IgG titer with increased all-cause mortality. Some results indicate the relationship of serostatus with increased mortality [11], whereas others did not confirm it [2].

AIM

The aim of this study was to assess the anti-CMV antibody titre, the genetic material of the virus in the plasma of elderly residents of nursing homes and the impact on the risk of death.

MATERIALS AND METHODS

Participants

The study protocol was approved by the Bioethics Committee at the Medical University of Warsaw (No. KB/13/2015).

The study was conducted at the nursing home in Warsaw, Poland. All residents were advised of the purpose of the study and gave their informed-, free-, written-consent to participate in the study. Persons having a pacemaker, aged below 65 and persons whose mental condition did not allow them to grant an informed consent were excluded from the study. The investigated group comprised 202 residents, 157 women (77.72%) and 45 men (22.28%). The mean age of the subjects was 82.16 ± 8.57.

Blood samples were collected between April and October 2015. The participants were observed for 1,095 days (3 years) between 2015 and 2018. During this period, 126 (62.37%) residents study participants died.

The planning, conduct and reporting of the study was in line with the Declaration of Helsinki, as revised in 2013.

Analytical methods

Determination of the genetic material of the virus

Plasma was obtained from peripheral blood drawn with the use of EDTA. The EDTA-treated plasma samples were centrifuged at 2000×g for 20 minutes then stored at -80oC until further processing.

DNA was isolated with the use of EZ1 Virus Mini Kit (Qiagen, Germany) in BioRobot EZ1 (Qiagen, Germany) according to the manufacturer's instructions. After DNA was verified with the use of the ND-1000 instrument (NanoDrop Technologies, Wilmington, Delaware, USA), nucleic acid was stored at -80°C. Real-time PCR was carried out using the ABI 7500 instrument (Applied Biosystems, Waltham, Massachusetts, USA). The CMV expression was measured using the Cytomegalovirus (CMV) PCR Kit from GeneProof (GeneProof, Brno, Czech Republic) according to the manufacturer's instructions.

Antibody titre determination

Serum was obtained from venous blood drawn into test tubes without anticoagulant agents. Blood was centrifuged for 10 minutes at 2 000 rpm in order to separate the serum from the clot.

The levels of CMV IgG antibodies in the serum were measured using the ARMITECT i1000 SR instrument (Abbott Laboratories, Abbott Park, Illinois, USA) by way of the Chemiluminescent Microparticle Immunoassay (CMIA) using the ARCHITECT CMV test (Abbott Laboratories, Abbott Park, Illinois, USA) according to the manufacturer's instructions.

The persons with IgG ≥6 AU/ml were considered CMV seropositive, whereas persons with IgG <6 AU/ml were considered seronegative.

Statistical analysis

All statistical analyses were carried out using the R statistical package, version 3.6.0. Statistical tests were used to identify significant relationships. Due to the number of residents in the individual groups, the Chi-squared test or the Fisher's exact test were used for categorical variables. The Mann-Whitney U test was used for continuous variables. Survivability curves were plotted using the Kaplan–Meier estimator. The assumed level of significance was $p<0.05$.

RESULTS

No genetic material of the CMV was found in the studied group of the residents.

The results of the determination of the antibody titre broken down by sex are presented in Table 1. In the whole investigated group (n=202), 15 residents were seronegative (n=15, 7.4%), while 187 residents were seropositive (n=178, 92.6%).

Tab. 1. Mean±SD of CMV IgG antibody in serum categorized into women or men and seronegative (CMV IgG < 6 AU/ml), (N=15) or seropositive (CMV IgG ≥ 6 AU/ml) (N=187) groups

CMV status	Seronegative (N=15)		Seropositive (N=187)		Seropositive IgG < 250 AU/ml (N=80)		Seropositive IgG ≥ 250 AU/ml (N=107)	
Division of patients	all patients		all patients		all patients		all patients	
	Women (N=10)	Men (N=5)	Women (N=147)	Men (N=40)	Women (N=57)	Men (N=23)	Women (N=90)	Men (N=17)
Mean ±SD CMV IgG [AU/ml]	1.13±1.26		222.78±46.48		186.38±52.29		250±0.0	
	1.21 ±1.45	0.98 ±0.70	226.99 ±40.57	207.32 ±61.20	190.66 ±45.71	175.77 ±64.60	250 ±0.0	250 ±0.0

Tab. 2. The rate of deaths among groups: seronegative, seropositive < 250 IgG [Au/ml], and seropositive >250 IgG [Au/ml], and among groups seropositive < 250 IgG [Au/ml] and seropositive > 250 IgG [Au/ml]

	parameter	survived	deceased	test	p value
Groups: Seronegative vs. IgG < 250 Au/ml and vs. IgG > 250 Au/ml	Seronegative	46.7% (N=7)	53.3% (N=8)	Pearson's Chi-squared test	0.56
	Seropositive < 250 IgG [Au/ml]	33.7% (N=27)	66.3% (N=53)		
	Seropositive > 250 IgG [Au/ml]	39.2% (N=42)	60.7% (N=65)		
Groups: IgG <250 Au/ml vs. IgG >250 Au/ml	Seropositive < 250 IgG [Au/ml]	35.8% (N=34)	64.2% (N=61)	Pearson's Chi-squared test	0.72
	Seropositive > 250 IgG [Au/ml]	39.3% (N=42)	60.7% (N=65)		

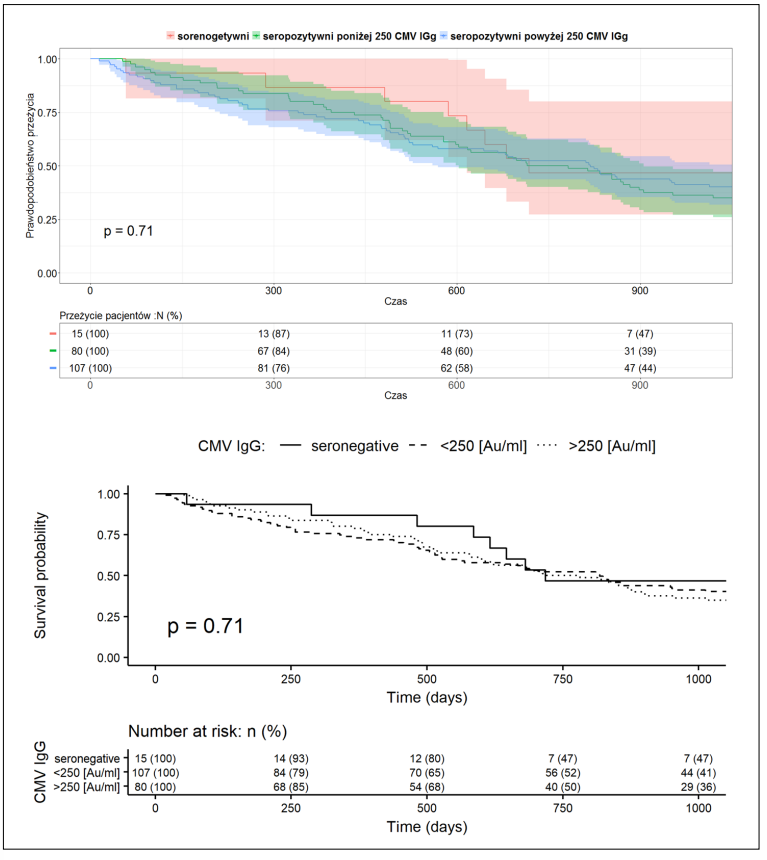

Fig 1. Kaplan-Meyer survivability curves in groups: seronegative, seropositive < 250 IgG [Au/ml], and seropositive > 250 IgG [Au/ml] in 3 years (900 days) observational period

Due to the fact that the IgG titre over 250 Au/ml is the limit of quantification of the test determined by its producer for persons with an antibody count exceeding 250 Au/ml, the measure was assumed to be 250 Au/ml.

The mean IgG titre among residents that survived (n=76) in the whole study group was 201.8 (SD 79.58) and among residents that deceased (n=126) was 209.06 (SD 69.78). U-Mann Whitney test did not reveal statistically significant difference between the group of deceased and surviving residents (p=1). However, due to the fact, that the measured titre 250 Au/ml, which is the limit of quantification of the test, these results are not fully valuable. Therefore, the same test was conducted among seropositive residents with IgG titre < 250 Au/ml. The mean IgG titre in this group among residents that survived (n=34) was 142.24 (88.22) and among residents that deceased (n=61) was 165.43 (79.96). The U-Mann Whitney test also did not reveal the statistically significant difference between the group of deceased and surviving residents (p=0.17).

To assess the risk of deaths among groups: seronegative, seropositive < 250 IgG [Au/ml], and seropositive > 250 IgG [Au/ml], and among groups seropositive < 250 IgG [Au/ml] and seropositive > 250 IgG [Au/ml], Pearson's Chi-squared test was conducted. No differences between selected groups were revealed. The results are shown in Table 2.

Then Fisher test was applied to assess the risk of death among selected groups: seronegative vs seropositive (p=0.58), seronegative vs. seropositive < 250 IgG [Au/ml] (p=0.38), seronegative vs seropositive > 250 IgG [Au/ml] (p=0.59), seropositive < 250 IgG [Au/ml] vs. seropositive > 250 IgG [Au/ml] (p=0.45). The odds ratio (OR) for the death in the listed above groups was, respectively: (OR: 1.5, 95% Cl: 0.52–4.31), (OR: 1.72, 95% Cl: 0.56–5.24), (OR: 1.35, 95% Cl: 0.46–4.01), and (OR: 0.79, 95% Cl: 0.43–1.44). The presented results indicate that there was the chance that the risk of death is slightly higher only in the seropositive group > 250 IgG [Au/ml] comparing to seropositive group < 250 IgG [Au/ml].

Survivability curves in groups: seronegative, seropositive < 250 IgG [Au/ml], and seropositive > 250 IgG [Au/ml], plotted using the Kaplan–Meier estimator are shown in Fig. 1. There were no differences between the selected groups (log-rank test, p=0.71).

DISCUSSION

In the present study we did not find genetic material of the CMV in the plasma of the studied group of the residents, what was in line with the previous studies [12, 13]. Even though the standard procedure is carrying out tests for presence of the CMV virus in the cells, in the work a decision was made to carry out the test for presence of DNA in the plasma and not the cells. This was done because, the CMV virus is also released to the plasma, especially under reactivation [14]. There are literature reports that suggest it is not the viral DNA, but rather the anti-CMV antibodies that are detected in the serum of immunocompetent residents that may be connected with the viral burden of these individuals [15]. Lack of reactivation of DNA virus may result from the selection of a group comprised of very old residents.

In this study, 92.57% of residents were positive for CMV; these data are comparable to seroprevalence data from Portugal, which reached 77% in the population ranged between 2 and above 65 years of age, and in the oldest group, e.g. above 65 years of age, seropositivity was observed in 95.65% of participants [16]. In Sweden the seropositivity was observed in 83% of participants of the study, while the mean age was 62.7 years, e.g. the participants were 20 years younger comparing to participants of our study [17]. Populations in France have lower CMV seroprevalence (41.9%), however the age of participants ranged from 15 to 49 years of age [18]. The available studies suggest that the percentage of residents infected with CMV increases with age, reaching a very high level in the studied group of residents [16]. In addition, the study group exhibited multiple morbidities. A high level of CMV infection is also a result of a relatively low socioeconomic status of residents that remained in nursing homes, similar to the status in some other countries [3].

The risk of death in the group of seronegative residents and in the group of seropositive residents was similar. The antibody titre was not found to influence the risk of death in the group of residents that were included in this study during the 3-year observation period. This observation was in line with the study of Mathei et al. [2]. However, other studies reported contrary results [19, 20]. The exact biological mechanisms by which CMV may impact health are still under investigation, but a growing body of evidence suggests that subclinical reactivation of the virus (throughout life) triggers clonal expansion of CMV-specific memory T-cells, ultimately contributing to age-related declines in immune function and increased levels of inflammation [2]. There are many possible mechanisms by which CMV infection may increase the risk of death. This may be related to the frequency and degree of CMV-reactivation, although these parameters are poorly explored in the elderly. CMV is a candidate causative agent for this reactivation as it chronically infects people throughout their life [21]. These mechanisms are mostly related to a very strong and extensive immune response towards CMV, which occurs after infection and which can spread in elderly residents very intensely [21]. Chronic infection by CMV engages a substantial fraction of T cells in infected people and demonstrates that such a phenomenon is massively amplified in the oldest residents, despite the well-demonstrated age-related dysfunction of anti-CMV CD8 T cells. The accumulated evidence clearly shows that cellular immunity plays a major role in the control of primary CMV infection as well as in the establishment and maintenance of latency [21].

Given that approximately 80% of individuals aged 65 years or older are seropositive for CMV [1], it is unlikely that infection serostatus alone will explain disease and mortality outcomes. Measuring antibody response, on the other hand, may capture subclinical reactivation of CMV and therefore, may be more relevant for examining CMV-induced inflammation and immune system dysfunction than seropositivity alone [5, 20].

We hypothesise that many of the oldest persons represent a phenotype that is less susceptible for the detrimental effects of CMV because of their capacity to strongly control the infection and thereby, to prevent reactivation. However, some will eventually fail to contain the virus because of an exhaustion of the immune system that causes the infection to reactivate and anti-CMV IgG titres to increase [2].

Given these qualities are nearly ubiquitous in CMV infection, it is not surprising that correlations between the mere fact of CMV infection and mortality are hard to establish.

The presented results are of great importance to nurses, especially working in nursing homes. Older people, due to the decrease of the function of immune system, known as immunosenescence, are especially susceptible for infection. Usually low economic status and high density of inhabitants increase the risk of infection. Nurses must be aware of this phenomenon and its consequences in order to provide appropriate environmental condition including protection against infection.

CONCLUSIONS

In summary, we did not find that the anti-CMV antibody titre has any effect on the risk of death in the study group, however we measured the titre of CMV antibodies only once, at the beginning of the observational period, what could influence the results. Whether the anti-CMV antibody titres increase the risk of death among immunocompetent elderly residents remains unclear. The group in which the study was performed came from one urban environment and was small and homogeneous (limited to residents remaining in the nursing home). Therefore, it would be necessary to perform studies on larger and more diverse cohorts to reveal the influence of CMV infection on the mortality rate.

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