https://doi.org/10.31925/farmacia.2022.2.16 **ORIGINAL ARTICLE**

CYTOMEGALOVIRUS INFECTION AND CARDIOVASCULAR RISK IN A MONOCENTRIC ROMANIAN ADULT PATIENT GROUP

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Abstract

Cytomegalovirus (CMV) is a widely distributed herpes virus in human populations that rarely causes any symptoms to healthy individuals. The infection can lead to serious health complications and even death for immature and immunocompromised people. Multiple studies have suggested that CMV could be a risk factor for developing cardiovascular diseases even in healthy carriers. Atherosclerosis is the root cause of many diseases like stroke or cardiovascular-related pathologies like angina, ischemic heart disease, acute myocardial infarction and cardiovascular death. In this study, we aimed to demonstrate the link between CMV serostatus and cardiovascular parameters like left ventricle mass and cholesterol level in a cohort of 145 volunteers. Also, we researched the benefits of cholesterol-lowering drugs in the prevention of CMV induced atherogenesis. The study revealed that the severity of cardiovascular disease is associated with an increased CMV IgG titter.

Rezumat

Citomegalovirusul (CMV) este un virus herpetic răspândit pe scară largă în rândul populației umane, care rareori provoacă simptome persoanelor sănătoase. Infecția poate duce la complicații grave de sănătate și chiar la deces pentru persoanele imature și imunocompromise. Mai multe studii au sugerat că CMV ar putea fi un factor de risc pentru dezvoltarea bolilor cardiovasculare chiar și la purtătorii sănătoși. Ateroscleroza este cauza principală a multor boli, cum ar fi accidentul cerebral vascular sau patologii cardiovasculare precum angina pectorală, boala cardiacă ischemică, infarctul miocardic acut și decesul cardiovascular. În acest studiu, ne-am propus să demonstrăm legătura dintre serostatusul CMV și parametrii cardiovasculari, cum sunt masa ventriculului stâng și nivelul colesterolului într-o cohortă de 145 voluntari. De asemenea, am cercetat beneficiile medicamentelor care scad colesterolul în prevenirea aterogenezei induse de CMV. Studiul a arătat că severitatea bolilor cardiovasculare este asociată cu un titru crescut de IgG-CMV.

Keywords: Cytomegalovirus infection, cardiovascular disease, diabetes, atherosclerosis

Introduction

Cardiovascular disease is a global health burden regardless of all efforts in reducing risk factors like hypertension, smoking, high LDL-cholesterol levels, diabetes or obesity [36]. Nowadays, it is well-known that the main underlying process of cardiovascular disease is atherosclerosis, with chronic arterial wall inflammation as the key process in initial and progressive atherosclerotic lesions [32]. During the last decades, many infectious agents that may cause an arterial inflammatory status were investigated [9, 10, 23]. Of them, cytomegalovirus is the only one constantly linked to atherosclerosis, cardiovascular disease and vascular neurocognitive disorders [1, 12, 21, 22].

Human cytomegalovirus, a member of the *Herpesviridae* family, is highly prevalent worldwide. According to World Health Organization, CMV infection is a major cause of disease and death in immunocompromised people and the leading viral cause of congenital disabilities in the world [14].

CMV is a ubiquitous DNA virus, with a worldwide seroprevalence from 80% to 100%, preponderantly depending on the socio-economic status [2]. According to CDC, in the US, over 50% of adults will develop the infection by the age of 40. Also, a third of the children

younger than five years old are already infected with CMV [11].

Using various immune evasion mechanisms, CMV can establish latency in almost all types of cells and tissues [8]. Therefore, it has been linked to all stages of atherosclerotic lesions, from endothelial injuries to atherothrombotic events [1, 4, 13].

The virus was detected in the atherosclerotic plaque and stimulates the pro-inflammatory signals in the cell environment promoting activation of both endothelial cells and peripheral blood mononuclear cells (PBMC), increasing their susceptibility to infection and cardiovascular risk [5, 22, 38].

The cholesterol-lowering drugs like statins may be important during treatments for reactivations of CMV infection, especially in the elderly and patients with other associated diseases like diabetes, in order to decrease the associated cardiovascular complications [16, 34].

Moreover, severe cardiovascular adverse events are decreased by 50% in diabetic patients under the treatment with statins due to their pleiotropic effects including lipid-lowering, anti-inflammatory, antioxidant, antithrombotic and antimitotic effect [27].

The study aims to highlight the impact of chronic cytomegalovirus infection on health status and assess the cardiovascular risk in chronically infected patients and the importance of monitoring these patients for initiating the optimal therapeutic approach.

Materials and Methods

Study design

One hundred and forty-five patients, aged 20 to 79 years old, mean age 55.13 years, admitted to the Department of Internal Medicine of Colțea Clinical Hospital in Bucharest, Romania, from February to December 2016, for acute or chronic pathologies, with a known or unknown cardiac disease were enrolled in the study. All patients gave informed consent, and the study was approved by the Ethics Committee of Colțea Clinical Hospital. At enrolment, each patient filled out a questionnaire on risk factors for cardiac disease (cigarette smoking, alcohol, diabetes, obesity, serum cholesterol levels and family risk factors) and medication use. The inclusion criteria were the confirmed presence of chronic infection with cytomegalovirus. The exclusion criteria were paediatric population and actual diagnosis or history of neoplasia, autoimmune disease, hepatitis B or C virus infection, HIV infection, Epstein Barr virus infection and sepsis.

All patients carried out a physical examination with arterial blood pressure measurement, cholesterol profiles evaluation using an Ortho Clinic Diagnostics automated dry biochemistry analyser and cardiac ultrasound evaluation using a Siemens Acuson X150 ultrasound device.

Left ventricular mass (grams) and left ventricular mass indexed to total body surface area were determined using the Devereaux modified formula:

$$0.8\{1.04[([LVEDD + IVSd + PWd]3 - LVEDD3)]\} + 0.6.$$

The severity of cardiac disease was assessed using AHA and ESC guidelines as well as New York Heart Association (NYHA) functional classification [26]: 0 = no cardiac changes; 1 = light cardiac changes – NYHA I; 2 = mild cardiac changes – NYHA II; 3 = moderate to severe cardiac changes – NYHA III – NYHA IV; and 4 = recent history or acute indication of minimally invasive or surgical procedures for cardiovascular disease.

Biochemical assays

Six mL of venous blood in a clot activator tube and 6 mL of venous blood on EDTA were collected from each patient. Anti-CMV IgG and IgM levels were measured in the "Ștefan S. Nicolau" Institute of Virology, Bucharest, Romania, using a quantitative enzyme immunoassay (DiaPro, Diagnostic Bioprobes SRL, Italy). According to the manufacturer's instructions, a sample with a reactivity over 0.5 UI/L was considered positive. The patients were divided into two groups based on the median value of reactivity for the CMV positive samples = low CMV IgG titters (0.5 - 5 UI/L) and high CMV IgG titers (> 5 UI/L).

Statistical analysis

Statistical analysis was implemented using the open-source software R (R version 4.1.1.) [29]. Numerical data sets were summarized by descriptive statistics, standard deviation and 95% confidence intervals. Bootstrapped Welch two-sample t-test was used to analyse the difference of the two numerical data sets not normally distributed [3]. The categorical variables were analysed by calculating percentages (%) and assessing associations with Pearson's chi-square test or by Fisher's exact test when the assumptions were not met. A part of the numerical data sets was transformed into qualitative variables (such as Age and BMI) and expressed by percentages (%). Statistical significance level was considered at alpha 5% ($p < 0.05$).

Results and Discussion

The study included 145 patients (43% male, 57% female) (Table I). All patients had a positive IgG anti CMV antibodies status. No acute CMV infection using IgM anti-CMV antibody status was detected. 25% of patients were under 50 y.o., 64% were 50 - 65 y.o. and 11% were over 65 y.o. 60% of patients presented at least light cardiac changes (Figure 1a), and 28% had diabetes (Figure 1b, 15% of them were insulin-dependent). 33.7% of patients were active smokers, and of them, 59% were heavy smokers with a consumption of more than 30 pack-year. 3.4% of patients declared chronic alcohol consumption of 60 to 100 g of alcohol/day.

Table I

Patients diagnosed with cytomegalovirus (CMV) infection

		Chronic CMV infected patients (n = 145)	
		Male % (n = 62)	**Female % (n = 83)**
Age	< 50 y.o.	26%	24%
	50 - 65 y.o.	65%	64%
	> 65 y.o.	10%	12%
Cardiac changes	0	35%	42%
	1	39%	46%
	2	6%	6%
	3	15%	6%
	4	5%	0%
Hypertension	0	37%	29%
	1	5%	7%
	2	21%	13%
	3	37%	51%
Diabetes	0	68%	76%
	1	27%	20%
	2	5%	4%
BMI	Overweight (> 25 kg/m^2)	76%	73%
	Normal weight (17 - 25 kg/m^2)	19%	22%
	Underweight (< 17 kg/m^2)	5%	5%
Smokers		45%	26.5%

Cardiovascular disease severity degree (0 = no cardiac changes; 1 = light cardiac changes – NYHA I; 2 = mild cardiac changes – NYHA II; 3 = moderate to severe cardiac changes – NYHA III; 4 = recent history or acute indication of minimally invasive or surgical procedures for cardiovascular disease – NYHA IV); Hypertension (0 = normal, 1 = grade I, 2 = grade II, 3 = grade III); Diabetes (0 = no diabetes, 1 = oral antidiabetics treatment, 2 = insulin-dependent treatment); BMI = body mass index

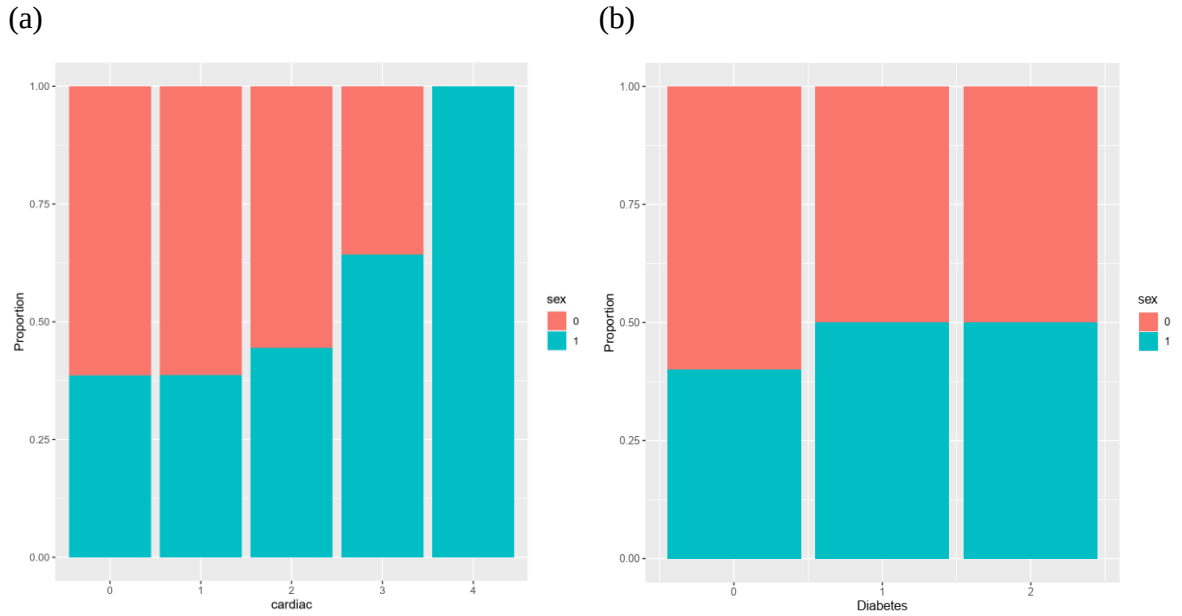

Figure 1.

Cardiovascular disorders (a) and diabetes (b) according to gender in patients with CMV infection (0 = female and 1 = male)

Most of the patients with cardiac changes ($p > 0.05$) and diabetes ($p < 0.01$) in our study were overweight, a condition that brings extra pro-inflammatory status and oxidative stress in the context of chronic CMV infection (Figures 2a and 2b).

CMV serostatus was assessed by determining the level of specific IgM and IgG antibodies in patients' blood. No patient presented IgM anti-CMV antibodies. Patients with a level of IgG antibodies over 0.5 UI/L were considered positive for chronic CMV infection. The patients were divided into two groups according to the blood level of antibodies as follows: group 1 included low anti-CMV IgG titters (0.5 - 5 UI/L), and group 2 had high anti-CMV IgG titters (> 5 UI/L).

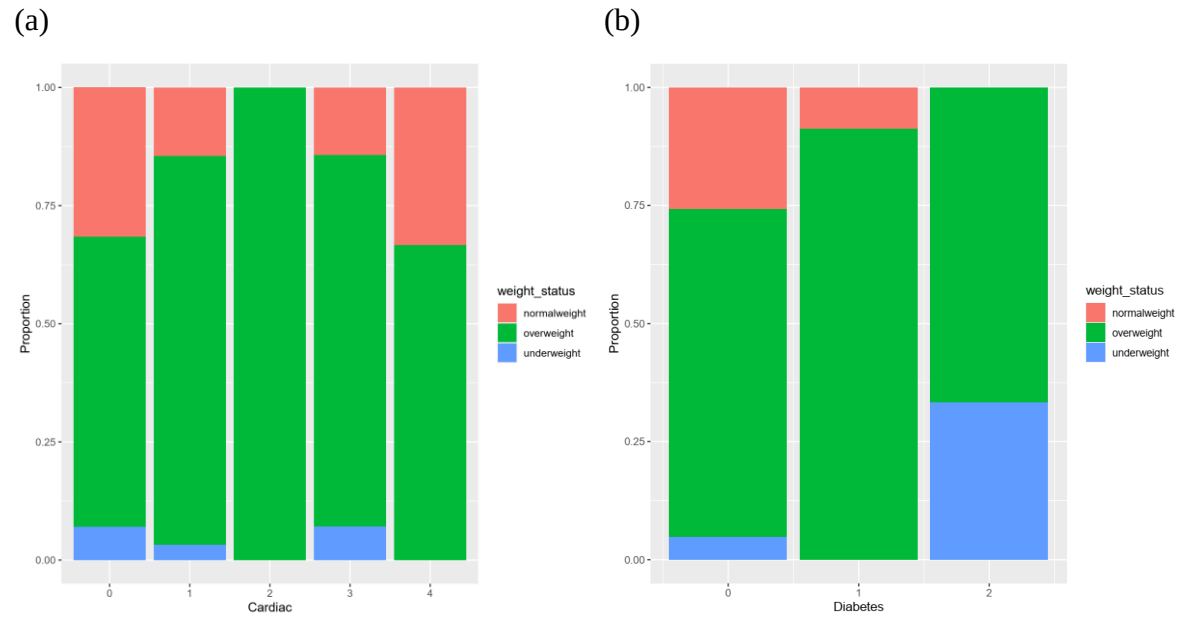

Figure 2.
Body mass index among the CMV infected patients diagnosed with cardiovascular disease (a) and diabetes (b)

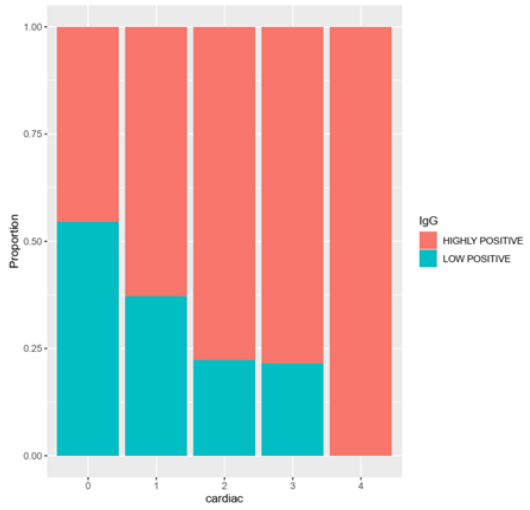

Figure 3.
The distribution of patients diagnosed with cardiovascular risk according to CMV IgG titter

We observed that the severity of cardiac disease (Figure 3) increased significantly with the CMV IgG titter ($p < 0.05$). Thus, 63% of NYHA I patients, 78% of NYHA II patients and 79% of NYHA III patients had high titters of IgG. All patients with NYHA IV cardiac failure or recent history or acute indication of minimally invasive or surgical procedures for cardiovascular disease belonged to group 2 with specific IgG titter over 5 UI/L. As for the left ventricle mass index alone and the severity of the cardiac disease in patients infected with CMV, the p-value of the test was < 0.001. As expected, the severity of cardiac disease was significantly correlated with the left ventricle mass with a correlation coefficient of 0.41, indicating a moderate to a strong relationship. Instead, we observed a low correlation (0.22) between the left ventricle mass index and specific IgG blood titter.

As a previous study showed, cardiac hypertrophy could be the consequence of long-term exposure to IL-6, an interleukin produced in larger amounts during CMV reactivation in chronic infection [7]. The reactivation of the CMV within the heart leads to altered cellular responses, which trigger the proliferation of endothelial and smooth muscle cells. The pro-inflammatory response helps CMV reactivation, causing a vicious circle that worsens the cardiovascular tissue's negative effects [6]. As previously mentioned, atherosclerosis is the main cause of cardiovascular disease, a complex process based on two key factors: inflammation and lipid accumulation within arterial intima [24]. The atherosclerosis risk is known to be higher in patients diagnosed with cytomegalovirus infection [19]. CMV infection could be an underlying cause that enhances the formation of atheroma plaque and the progression of atherosclerosis through different mechanisms, most of them aiming arterial wall inflammation. Once infected, vascular endothelial cells are prone to integrity disruption, dysfunctional metabolism and even apoptosis, a damaging process mediated by viral proteins IE84 and IE72. Moreover, antibodies antiviral proteins UL122 and US28 can activate growth factors, cytokines, and adhesion molecules, which are connected to the evolution of atherosclerosis. Usually, endothelium displays anti-atherosclerosis proprieties by minimizing thrombosis and inflammation. Yet, some research demonstrated the opposite in CMV-infected vascular endothelium, meaning a pro-coagulant response and an increased aggregation and adhesion of thrombocytes and an

upregulation of inflammatory factors like IL-6, IL-8 and RANTES [38].

Table II

The variation of blood parameters according to the presence of cardiovascular risk among CMV patients

		Mean ± SD
Total cholesterol	-CV	185.69 ± 43.7
	+CV	189.37 ± 53.8
HDL - cholesterol	-CV	52.95 ± 25.4
	+CV	47.41 ± 14.3
LDL - cholesterol	-CV	110.76 ± 35.4
	+CV	110.11 ± 44.4
Triglycerides	-CV	111.36 ± 65.8
	+CV	160.26 ± 77.8*

-CV/+CV = absence/presence of the cardiovascular risks; * P (Wilcoxon) < 0.05

To achieve both key factors in atherosclerosis, we also evaluated the blood lipid profiles of all patients included in our study (Table II).

In the case of total cholesterol, HDL-cholesterol and LDL-cholesterol blood levels, there were no significant statistical differences between the patient group with at least one cardiovascular risk and the group with no cardiovascular risk reported (Table II). Still, in both groups, the LDL-cholesterol blood levels were higher than 75 mg %, which was a concerning aspect, especially for the patients with active CMV infection and already installed cardiovascular disease.

The treatment with statins was considered for 41 patients included in the study. The blood level of total cholesterol ($p < 0.01$) and LDL-cholesterol ($p < 0.01$) was significantly decreased among patients treated with statins compared to those who did not receive cholesterol-lowering medications (Figure 4).

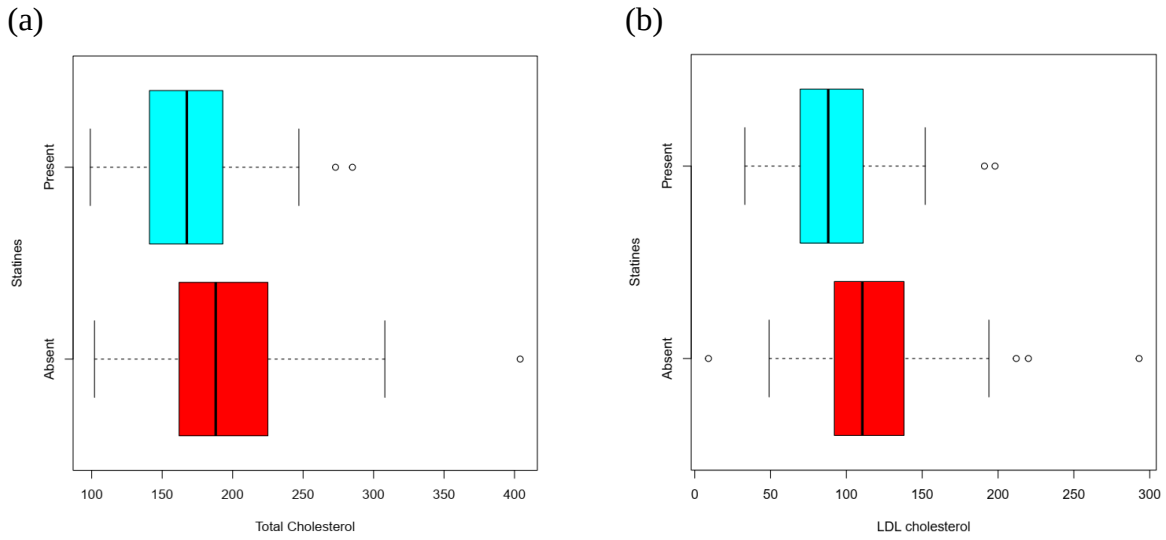

Figure 4.

The impact of the statins treatment on the blood level of total cholesterol (a) and LDL-cholesterol (b) in patients diagnosed with CMV infection

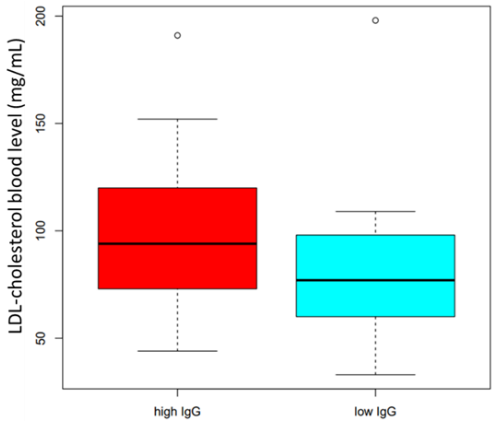

Figure 5.

LDL-cholesterol level according to IgG CMV antibodies titter (high IgG > 5 UI/L, low IgG < 5 UI/L) in patients treated with statins

In the case of the treatment with statins, the CMV infected patients with a high titter of IgG antibodies showed a slight increase of mean LDL-cholesterol level but with no significant differences compared to the patients with low IgG CMV titters (Figure 5). The treatment with statins, used primarily for their hypolipaemiant role, has been shown through different studies also having beneficial effects on reducing CMV titters in endothelial cells [16, 17, 34]. Comparable with ganciclovir antiviral activity, statins were potent also on ganciclovir-resistant CMV strains [28]. The antiviral activity was observed in atorvastatin, simvastatin and fluvastatin, which seems to curb the replication chain of the virus by reducing the formation and activity of CMV's IE antiviral protein antigens [23].

During the acute infection, CMV targets the endothelial cells, smooth muscle cells and the immune system cells. CMV is transmitted by blood and contaminated

secretions (saliva and urine) *via* intercourse, organ transplantation, transplacental or through lactation from mother to new-born [33]. Once infected, a healthy organism is never cured of CMV. The virus persists as a latent infection and can reactivate anytime if the immune system is impaired [31]. The virus is kept latent mainly due to the action of T-cells, but other immune cells like NK, monocytes and macrophages are also involved [20]. CMV infection acts through inflammatory and immunological mechanisms. It has been stated that cytomegalovirus affects the host's immune system as no other existent human pathogen in terms of latency and enlargement of immune cells populations. The immune system reacts by boosting the number of memory T cells phenotypes and NK cells for CMV-seropositive persons. This phenomenon is then followed by inflammatory and cytotoxic processes [30]. Depending on the period of life when a person acquires CMV, the virus can have both: favourable or deleterious effects on the host's immunity. Intrauterine infection usually ends with life-long neurologic squeals for new-borns and it was even associated with chances of developing acute lymphoblastic leukaemia later in life. For children and young adults, it was demonstrated a correlation between CMV-seropositivity, a better response to influenza vaccine and protection against other pathogens [30]. For elderly people, the presence of CMV in their system is mostly associated with cardiovascular disease and less with other pathologies like infections and cancer [25]. The long-term infection itself could lead to the production of pro-inflammatory cytokines like IL6 and TNF-alpha, which are also mortality related factors. A large titter of antibodies may result from the multiple reactivations of the CMV, which is, in fact, a path to a much more important inflammatory response that could damage vascular endothelium and the cardiovascular system overall [35].

Multiple studies have been suggesting that CMV could be a risk factor for the development of cardiovascular diseases, especially in the progress of atherosclerosis. Some studies did not succeed in showing any relationship between the presence of CMV in vascular endothelium and atherosclerotic plaque and almost no difference when compared the CMV in healthy individuals' vascular cells with those who have atherosclerosis [37]. Other studies, on the contrary, demonstrated a higher replication of the virus in the atherosclerotic plaques for those with acute coronary disease and a link between familial history for cardiovascular syndromes and the spreading of CMV in the vascular endothelium [18]. Besides atherosclerosis, CMV is suspicious as being a risk factor for the development of arteroscleroses and its negative effects like lower flexibility of arteries, elevated pulse wave velocity and high blood pressure, especially because all these are associated with an increased proportion of CMV specific T cells [25].

CMV can also stimulate other pathogens' effects. HIV/CMV coinfection is the leading cause for CMV retinitis and other serious non-AIDS pathologies, including gastrointestinal, neurological, hepatic syndromes etc. The coinfection is very common and associated with increased inflammation. Patients under antiretroviral therapy seem to have better therapy outcomes and resistance to adverse effects if they are only seropositive for HIV, but not for CMV [15].

Conclusions

The infection with cytomegalovirus increases the risk of cardiovascular disease and other associated disorders like diabetes among patients. The early diagnosis is important for patient health status evolution. The administration of cholesterol-lowering medication for an LDL-cholesterol target < 75 mg/mL should be considered for CMV infected patients in order to reduce arterial wall inflammation and regulate LDL-cholesterol homeostasis as well as to specifically control anti-CMV antibodies titters.

Conflict of interest

The authors declare no conflict of interest.

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