THE SEROLOGICAL EVALUATION OF THE CYTOMEGALOVIRUS (CMV) AND *CHLAMYDIA PNEUMONIAE* INFECTIONS IN PATIENTS WITH CARDIOVASCULAR DISEASES (CVDs)

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ABSTRACT

Serological evidences of CMV and *Chlamydia pneumoniae* infectious were highly prevalent in all human population samples. There was a limited epidemiological data relating these infections to myocardial ischemic disease (IHD) and future risks in patients with acute coronary syndrome (ACS) and atherosclerosis.

The aim of our study were to evaluated: (i) the serum levels of IgG antibodies to CMV and *Chlamydia pneumoniae* in patients with different CVDs and healthy controls; and (ii) the risks related to persistent CMV and chronic *C. pneumoniae* infections for late cardiac events in the general population.

Plasma specimens were collected from 93 patients (55 men and 38 women aged 41 - 85) who have been hospitalized in IVth Cardiology Clinic affiliated to Medical University - Sofia. They were analysed for IgG antibodies to CMV and *C. pneumoniae* by ELISA. The patients were divided into 4 groups: 1) chronically IHD; 2) ACS; 3) stable angina pectoris (AP); and 4) healthy controls.

52 cases (94,5 %) and 23 cases (60,5 %) were seropositive for CMV. Of the 93 patients, 24 (25,8%) had positive tests for anti-*C. pneumoniae* IgG antibodies. Optical density, an indicator of CMV antibody titre, was higher in the patients with ACS than in other groups: median 36 NTU/ml (range 4 – 43 NTU/ml) versus 25 – 26 NTU/ml in IHD, stable AP and in controls. Indeed, the proportion of cases with positive IgG antibodies to CMV was not statistically different between the 4 groups (94,5% in ACS, 90,2% in chronically IHD, 90,5% in stable AP and 92,4% in the controls. However, only serum concentrations of IgG antibodies to *C. pneumoniae* were significantly associated with acute coronary events, smoking behaviour and hypertension. Among controls, seropositivity was not associated with age and gender.

These observations linking that CMV and *C. pneumoniae* infections may be implicated in ACS, but is unlikely to be a strong risk factor for development of myocardial infarction, chronically IHD and stable AP.

***Key words**: Cytomegalovirus (CMV), Chlamydia pneumoniae, acute coronary syndrome (ACS), angina pectoris (AP), myocardial ischemic disease (IHD), seroepidemiology*

Introduction

Cardiovascular diseases remained the most significant threat to the health of human population all over the world. At present, known risk factors for the developing of atherosclerosis such as genetic predisposition, hypercholesterolemia, hypertension, smoking, and diabetes mellitus do not clarify all cases of the coronary heart disease, which suggested that there were other unidentified factors (1). The ability of various infectious agents to developed chronic infections and their secreted products to altered vascular cells may play a crucial role in the pathogenesis of cardiovascular diseases (2 - 4).

In recent years, various studies have indicated that both ubiquitous pathogen cytomegalovirus (CMV) (5 – 8) and *Chlamydia pneumoniae* (8 – 14) were associated with atherosclerosis. This

hypothesis was based on seroepidemiological studies (5, 8, 9, 12 - 15), evidence of viral nucleic acid in atherosclerotic plaques (16), and the development of animal models of atherosclerosis in normocholesterolemic conditions (17). It was not clear whether the infectious burden could explain the role of inflammation in cardiac instability and atherosclerosis or not.

General, in immunocompetent patients, primary CMV infection typically runs as mononucleosis-like syndrome (18). To the contrary, CMV disease (reactivation of previously latent infection or newly acquired infection) frequently developed in patients immunocompromised by HIV infection (19, 20), solid-organ or bone marrow transplantation (21 - 23), as well as other disorders. In patients co-infected with HIV, CMV infection leaded to progression to AIDS and eventually death (20). In addition, cytomegalovirus has emerged in recent years as the most important cause of congenital infection in the developed world, commonly leading to mental retardation, spastic paralysis, hepatosplenomegaly, thrombocytopenia and developmental disability (24 - 26). CMV had also been associated with some chronic diseases of aging, including physical impairment, cognitive decline and cancer (27). The strongest evidence of a pathogenic role of CMV in atherosclerosis, however, was in cardiac transplantation (28, 29).

Similarly, there was increasing evidence that *Chlamydia pneumoniae*, ubiquitous pathogen that causes acute respiratory disease, may play a significant role in atherogenesis. Exposure to *C. pneumoniae* was common, with 50% of individuals seropositive by 20 years of age and approximately 80% by 80 years old (30). This agent generally caused mild upper respiratory tract infections, which range in severity from asymptomatic disease to acute and chronic diseases such as pneumonia, pharyngitis, bronchitis and sinusitis (30). *C. pneumoniae* has been associated and with coronary and carotid artery disease in seroprevalence epidemiological studies (31, 32).

Increasing evidence supported the thesis that atherosclerosis was based on a chronic inflammatory process (33), which elevated interleukin (IL)-6 levels (34) and cytokine production (34 - 36). Reports of such associations had raised the possibility that anti-infective treatments might be able to prevent cardiovascular disease. Mediated by this systemic inflammatory process, CMV and *C. pneumoniae* infections might contribute to the atherosclerotic and atherothrombotic processes.

On the basis of this experimental experience and the hypothesis that the inflammatory immune response might provide the link between infection and progression of atherosclerosis, we investigated the serum levels of IgG antibodies to CMV and *C. pneumoniae* in patients with different CVSs and the risks related to persistent infection for late cardiac events.

Materials and Methods

Patient characteristics

The study was conducted at the National Centre of Infectious and Parasitic Diseases – Sofia (Bulgaria) in collaboration with Sofia Medical University. The study group was 93 patients (55 men and 38 women) who had been hospitalized in IVth Cardiology Clinic affiliated to Medical University - Sofia. Details of their clinical history were recorded on admission. All patients aged from 41 to 85 years and were divided according different CVDs, such as chronically myocardial ischemic disease (IHD), acute coronary syndrome (ACS), stable angina pectoris (AP) and healthy controls.

We defined a patient as having IHD if there was any angiographic evidence of atherosclerosis, including plaquing in any segment of the epicardial coronary tree, chest pain, pulmonary embolism, hyperlipidaemia, hypertension and the family history

Patients with angina-type chest pain associated with nausea and sweating and ST segment depression or negative T waves, were included in the ACS group.

The diagnosis of stable AP was based on WHO criteria as angiographic evidence of atherosclerosis in their epicardial coronary tree and those without as healthy controls who were admitted to Cardiology Department for elective coronary angiography.

No patient admitted to the study had a myocardial infarction within the previous three months. All the patients gave their informed consent before being included in the study. In generally, study patients were of Bulgarian nationality and lived in the Sofia area.

CMV and Chlamydia pneumonia serological studies

Blood samples were taken on admission by venipuncture from each patient and tested for serum immunoglobulin G (IgG) antibodies to CMV and to *C. pneumoniae*. Blood was centrifuged at 4000*g* for 10 min and serum was aliquoted and frozen at -80^{o}C until analyzed. Serum IgG titres were measured using commercial enzyme-linked immunoassay (ELISA) kits (for anti-*C. pneumonia*e IgG, EUROIMMUN, Germany and for anti-CMV IgG, NovaTech, Germany). Both were a semi-quantitative tests, in which results are expressed as a ratio of extinction value of the control or patient sample over extinction value of the calibrator (ratio = extinction of the control or patient sample/extinction of the calibrator). In EUROIMMUN test ratio ≥1.1 was taken as positive. With NovaTech test samples were considered positive if the absorbance value was higher than 10% over the cut-off (value > 11 NTU). Samples were negative if the absorbance value was lower than 10% below the cut-off (value < 9). Quantitative values of antibodies for both tests were obtained from the standard curve by point-to-point plotting of extinction values against the corresponding units.

Statistical analysis

Tests were done in triplicate and in two separate experiments. Categorical data were analyzed by the χ^2-test. Variables with control distribution are presented as mean and standard deviation (SD). A value of $p < 0,05$ was considered statistically significant.

Results and Discussion

Of the 93 study patients, 55 (59 %) were man and 38 (41 %) women. All patients aged between 41 and 85 years. Patients were divided generally into 4 groups: 1) 22 (23,7 %) patients with IHD; 2) 30 (32,6 %) – with ACS; 3) 19 (20,4 %) patients with AP and 4) 22 (23,7 %) patients in the control group (Fig. 1).

Figure 2. The proportion of cases with CMV positive antibody titre

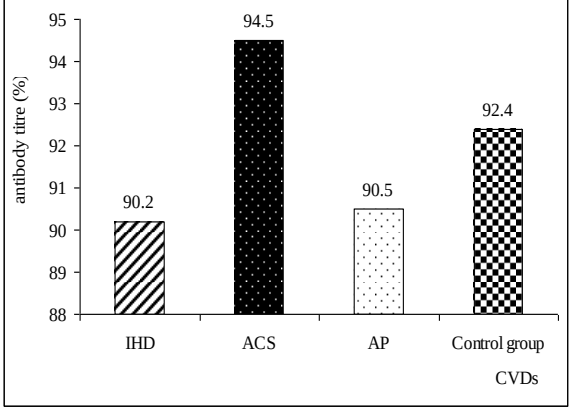
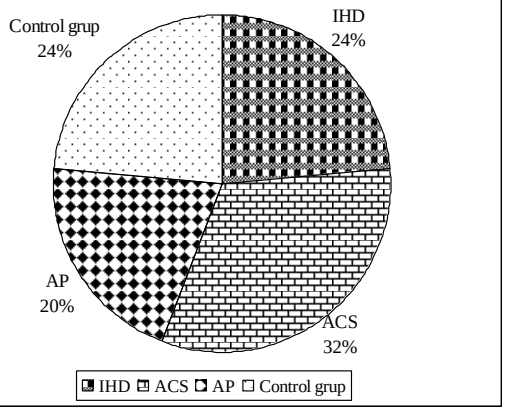


Figure 1. The distribution of patients with different CVDs



Of all patients with CVDs studied 52 cases (94,5 %) and 23 cases (60,5 %) had anti-CMV IgG antibodies. Optical density, an indicator of CMV antibody titre, was higher in the patients with ACS than in other groups: median 36 NTU/ml (range 4 – 43 NTU/ml) versus 25 – 26 NTU/ml in IHD, stable AP and in controls. The ratio relating CMV seropositivity to incidents with ACS was 5,4 (32,6 % from all cases with CVDs). However, the proportion of cases with CMV positive antibody titres was not statistically different between the 4 groups (94,5 % in ACS, 90,2 % in

chronically IHD, 90,5 % in stable AP and 92,4 % in the controls (Fig. 2). Among controls, seropositivity was not associated with age and gender.

Of the 93 patients, 24 (25,8 %) had positive tests for anti-*C. pneumoniae* IgG antibodies. Fifteen (62,5 %) were man and 9 (37,5 %) were women. This coincided in published data according which *C. pneumoniae* antibody seroprevalence rates tend to be higher in men than in women (37), suggesting that men were more susceptible to *C. pneumonia* infection than women. Iron levels were in general higher in men than in women (38) and were known to be essential for developing of microbial infection. However, only serum concentrations of IgG antibodies to *C. pneumoniae* were significantly associated with acute coronary events, smoking behaviour and hypertension. Several limitations must be considered in interpretation of these findings. We used the presence of IgG antibodies (for *C. pneumoniae*, IgG titer) measured on a single occasion late in life to characterize prior infection. This result may have had inadequate statistical power to detect very high risk of cardiac death associated with the presence of prior infection with *C. pneumoniae*. In addition, the data from this study may not be generalizable to young and middle-aged adults.

Conclusions

In this prospective study of a large, angiographycally defined patient group, we founded that the pathogens described in this study were unlikely to be strong predictors of risk of CVDs and future cardiac death. Probably, antibody titer-dependent connection of CMV and *C. pneumoniae* may be implicated in ACS, but is unlikely to be a strong risk factor for development of myocardial infarction, chronically IHD and stable AP. Our data were in a contrast with a various studies that these agents may be implicated in the pathogenesis of atherosclerotic disease and being associated with a bad prognostic clinical outcome. The study does not however completely exclude that CMV and *C. pneumoniae* infection or reactivation/reinfection plays any role in atherosclerotic disease of the coronary arteries. This was the first pilot study, which was done in Bulgaria regarding connection of CMV and *C. pneumoniae* and acute cardiac events among older adults. Several secondary prevention trials that examine the effect of macrolide antibiotic therapy for *C. pneumoniae* among adults with different CVDs are now in progress.

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