CLINICAL STUDY

Chlamydia pneumoniae antibodies and markers of inflammation in patients with cardiovascular diseases

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Abstract

***Background:* Chlamydia pneumoniae is suggested to be associated with cardiovascular diseases.**
***Objectives:* To study the presence of IgG, IgA anti-*C. pneumoniae* antibodies, interleukin-6 (IL-6), and C-reactive protein (CRP) as markers of previous *C. pneumoniae* infection and inflammation, in sera of patients with acute myocardial infarction (AIM), hypertension (HT), and coronary heart disease (CHD).**
***Methods:* Determination of these markers by ELISA method.**
***Results:* Proportion of samples containing both IgG and IgA antibodies as well as IL-6 was significantly higher in all groups of patients than in a control group. The CRP was significantly higher in patients with AIM and HT, however, in other patients, the proportion of positive samples depended on the chosen cut-off value.**
***Conclusions:* The results obtained indicate the feasibility of following chlamydial antibodies on higher number of serum samples extended to direct detection of *C. pneumoniae* in blood and vascular tissue *(Tab. 2, Ref. 24)*.**
Key words: *Chlamydia pneumoniae,* antibody detection, interleukin-6, C-reactive protein, cardiovascular diseases.

Chlamydia pneumoniae, formerly known as a TWAR agent, is an important cause of pneumonia and other acute respiratory diseases (1). During nineties, this small obligate intracellular bacteria was associated not only with other respiratory diseases, such as chronic obstructive pulmonary disease, asthma, and lung carcinoma, but also with other clinical entities, namely erythema nodosum, Reiter's disease, sarcoidosis, and atherosclerosis (2, 3, 4).

First evidence on the possible role of *C. pneumoniae* have given Finnish authors who have found significantly higher chlamydial antibodies in patients with chronic coronary heart disease and acute myocardial infarction than in healthy population (5). Subsequent seroepidemiologic studies suggested an association of IgG anti-*C. pneumoniae* antibody with both coronary (6) and carotid (7) atherosclerosis. In acute myocardial infarction, apart from a high prevalence rate of IgG and IgA-specific anti-*C.pneumoniae* antibodies also high concentrations of interleukin-6 (IL-6) were found (8). Low-grade infections, as one cause of the inflammatory reaction observed in atherosclerotic lesions and acute ischemic symptoms, was reflected by elevated levels of C-reactive protein (9). However, as summarized by Siscovisk et al (10), several prospective studies failed to demonstrate an association between the presence of *C. pneumoniae* antibody and myocardial infarction.

Though we have confirmed both prevalence (11) and incidence (12) of *C. pneumoniae* infection in our territory, data on the possible association with cardiovascular diseases are missing. Therefore, the aim of this study was to detect IgG and IgA specific anti-*C. pneumoniae* antibodies along with IL-6 and C-reactive protein (CRP) in serum of patients with acute myocardial infarction (AMI), coronary heart disease (CHD), and hypertension (HT), and to compare the obtained results with a control group with no overt cardiovascular problems.

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Acknowledgements. Statistical analysis performed by Dr. S. Wimmerová is highly appreciated.

Tab. 1. The presence of IgG, IgA anti-*C. pneumoniae* antibodies, IL-6, and CRP in study groups of cardiovascular patients and control subjects.

Group examined	Chlamydial antibodies IgG	IgA	IL-6	CRP	Total No. examined
AMI	20 (76.9%)	17 (65.4%)	25 (96.2%)	13 (50.0%)	26
	p = 0.025	p = 0.055	p<0.00	p<0.001	
	3.0 (1.1-8.3)*	2.4 (0.9-5.9)*	18 (2.3-140.0)*	36 (7.3-178.6)*	
IHD	18 (75.0%)	16 (66.7%)	16 (66.7%)	2 (8.3%)	24
	p = 0.044	p = 0.049	p>0.05	p>0.05	
	2.7 (1.0-7.6)*	2.5 (1.0-6.5)*	1.4 (0.6-3.8)*	3.3 (0.4-24.6)*	
HT	24 (92.3%)	19 (73.0%)	21 (80.1%)	5 (19.2%)	26
	p<0.001	p = 0.011	p = 0.031	p = 0.012	
	10.8 (2.4-48.9)*	3.4 (1.3-9.0)*	3.0 (1.1-8.9)*	8.6 (1.6-47.4)*	
IHD + HT	19 (82.6%)	17 (73.9%)	21 (91.3%)	4 (17.4%)	23
	p = 0.009	p = 0.012	p = 0.002	p = 0.027	
	4.3 (1.3-13.8)*	3.5 (1.3-9.9)*	7.6 (1.6-34.7)*	7.6 (1.3-44.5)*	
Patients together	81 (81.8%)	77 (77.8%)	83 (83.8%)	24 (24.2%)	99
	p<0.001	p<0.001	p<0.001	p<0.001	
	4.0 (2.0-8.1)*	4.4 (2.3-8.4)*	3.7 (1.8-7.6)*	11.5 (2.6-50.5)*	
Control	39 (52.7%)	33 (44.6%)	43 (58.1%)	2 (2.7%)	74

Numbers and proportion (%) of positive of total number of examined sera.
*Odds ratio and confidence (95%) interval.

Material and methods

Blood was collected from 26 patients who had suffered 3–6 months ago from an AMI, 26 patients with HT, 24 patients with CHD, and 23 with both HT and CHD. Control group consisted from 74 "white collar" workers with no cardiovascular complains. Serum was separated within one hour after blood collection and kept frozen at -70 °C. Patients with AMI were 60–70 years old, other groups of patients 50–60 years old, and control group was 30–40 years old.

The presence of IgG and IgA specific anti-*C. pneumoniae* antibodies in serum was assessed using ELISA, SeroCP-IgG and SeroCP-IgA (Savyon Diagnostics Ltd, Israel) kits. Serum with an optical density ≥1.1 was considered positive. IL-6 was detected by IL-6 ELISA kit (Immunotech, France); with a positive value >3 ng/L. Detection of CRP was performed by US C-reactive Protein ELISA (Diagnostic Systems Laboratories, Inc., USA) as described, with a positive value ≥10 mg/L. All ELISA analyses were performed and calculated according to the manufacturers instructions.

For the statistical analysis, chí^2 test and Fischer exact test in contingency table was employed.

Results

As shown in Table 1, a significantly higher amount of positive samples was observed in 99 cardiovascular patients, i.e. the presence of IgG and IgA specific anti-*C. pneumoniae* antibodies in 79.8 % and 75.8 %, and of IL-6 and CRP in 83.8 % and 24.2 %, respectively, compared to the number of reacting samples in 74 control subjects that was 52.7 % for IgG, 44.6 % for IgA, 58.1 % for IL-6, and 2.7 % only for CRP ($p<0.001$ for all tested markers). When a 3 mg/L cut-off value of CRP was chosen (not included in Table 1), it was found in 72.7 % samples of patients in total, but in 45.9 % samples of a control group ($p<0.001$).

However, some differences were found also among groups of patients with different cardiovascular disease. Regarding IgG antibodies, the highest proportion (92.3 %) was noticed in the patients with HT, the lowest (76.9 %) in patients with AMI. Differences in serum IgA antibodies were not so marked, i.e. from 57.7 % in patients with AMI to 73.9 % in patients suffering from both HT and CHD. On the other hand, the highest proportion of serum IL-6 and CRP was found in patients with AMI (96.2 % and 24.2 %, respectively), the lowest in patients with CHD (57.7 % and 2.7 %). With a CRP cut-off value 3 mg/L (not included in Table 1), a significant proportion of positive samples was found in patients with AIM (96.2 %; p<0–001) and HT (73.1 %; $p<0.001$), but not in patients with CHD alone (62.5 %; $p=0.159$) and CHD associated with HT (56.5 %; $p=0.375$) as compared to the control group (45.9 %).

Regarding above mentioned markers, a comparison of positive samples in men and women revealed practically no or very low difference for IgG antibodies and CRP, but higher proportion of positive samples for IgA antibodies and IL-6 was observed in women, i.e. 86.0 % vs 75.0 % for IgA antibodies and 93.0 % vs 76.8 % for IL-6, respectively (Tab. 2).

Discussion

Anti-*C. pneumoniae* antibodies are spread worldwide in an adult population (13). In a collaborative study with WHO Reference Laboratory in London, in a micro-immunofluorescence test 43.0 % of men and 23.8 % of women sera, of more than a thousand samples tested, reacted with chlamydial strain IOL-207 (11). This was confirmed later on as *C. pneumoniae.*

Tab. 2. Numbers and proportion (%) of samples of men and women with cardiovascular diseases containing IgG, IgA anti-*C. pneumoniae* antibodies, IL-6, and CRP.

Gender	IgG	IgA	IL-6	CRP	Total No.examined
Men	43 (76.8%)	42 (75.0%)	43 (76.8%)	13 (23.2%)	56
	p>0.05	p = 0.038	p = 0.026	p>0.05	
	1.3 (0.5-3.2)*	3.3 (1.0-10.7)*	4.0 (1.1-15.2)*	1.2 (0.5-2.9)*	
Women	31 (72.1%)	39 (86.0%)	40 (93.0%)	11 (25.6%)	43

Number and proportion (%) of positive of total number of examined sera.
*Odds ratio and confidence (95%) interval.

Since repeated infections during life are common, higher proportion of positive samples can be expected in elderly. Though there are no universally accepted criteria for the diagnosis of chronic *C. pneumoniae* infection, IgA may be a marker for persistent *C. pneumoniae* infection (14). However, a lower frequency of AMI was observed in IgA positive patients (15).

As far as cardiovascular diseases are concerned, inflammation via response to antigenic or non-antigenic stimulation is commonly thought of as a putative mechanism of coronary heart disease (16). A potential role of a persistent bacterial infection in atherosclerosis, especially *C. pneumoniae,* was stressed (17), but other authors say it has not been proven and it is still controversial (18, 19, 20). Recent studies have shown that an increased seropositivity for *C. pneumoniae* was not significantly associated with death (21) and that inflammatory markers showed an independent and stronger correlation with carotid atherosclerosis compared to markers of *C. pneumoniae* infections (22).

In our pilot study was the proportion of samples containing both IgG and IgA anti-*C. pneumoniae* antibodies significantly higher in all cardiovascular patients as well as in those with different cardiovascular disease diagnosis compared to control group. When taking into account the possible increase of *C. pneumoniae* infections with age, it could be attributed also to higher age of patients than of control subjects. Similarly, IL-6 and CRP as markers of inflammatory responses have occurred more frequently in patients than in control subjects. It is not surprising that the highest proportion of positive samples containing CRP was observed in patients with AMI. The CRP is considered not only as an excellent inflammatory marker, but also as a direct participant in atherogenesis (23). In this connection, the cut-off value of this marker is also important, e.g. a CRP level of 10 mg/L can be proposed for the stratification of the risk of death, but 3 mg/L is a value to be used for long-term stratification of stable and unstable patients, if samples are taken at discharge (24).

The number of patients tested in our study was too low to speculate about the possible association of *C. pneumoniae* with cardiovascular diseases. However, the obtained results indicate that this association is not excluded. Further, extended serological studies as well as the use of methods directly detecting the agent in blood or vascular tissue, are needed. At the same time, more detailed analysis of CRP values in patients with defined cardiovascular diseases is necessary.

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