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The relationship between *Chlamydophila pneumoniae* IgG titer and coronary atherosclerosis

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Abstract

Background: *The role of* Chlamydophila pneumoniae *(CP) in the progression of atherosclerosis is controversial. Also no sufficient angiographic study is available about the impact of CP infection on severity and intensity of coronary atherosclerosis. We investigated the relation between CP IgG antibody titers and severity and intensity of coronary atherosclerosis*

Methods: *The study population consisted of 516 consecutive patients who underwent a coronary angiography. The group included 353 patients who had coronary artery disease; a control group included 163 subjects with angiographically proven normal coronary arteries.* Chlamydophila pneumoniae *IgG antibody titers were measured by an enzyme immunoassay method in all patients. Gensini scores and extent scores were used to evaluate the angiographic extent and severity of atherosclerosis.*

Results: *The mean value of IgG antibody titer was 44.3 ± 28.8 IU/mL in the patients and 39.8±27.4 IU/mL in the control group ($p = 0.14$). There was no statistically significant correlation between the Gensini scores, extent scores and CP IgG titers (Gensini score: $r = +0.103$, $p = 0.07$, extent score: $r = +0.110$, $p = 0.31$). When we grouped the patients as high (> 50 IU/mL) and low (< 50 IU/mL) IgG antibody titers, the number of diseased coronary arteries was higher in patients with high IgG antibody titers (respectively: 2.6 ± 1.1* vs. *2.2 ± 0.8, $p = 0.01$). While the Gensini score was significantly higher in patients with high IgG antibody titers (7.5 ± 4.0* vs. *6.17 ± 4.0, $p = 0.01$), the extent score did not change with IgG titers (29.8 ± 15.9* vs. *25.8 ± 15.4, $p = 0.08$).*

Conclusions: *In our study, we investigated the relation between CP infection and coronary atherosclerosis and found that CP IgG antibody titers are associated with the severity of coronary stenosis at higher antibody levels. However, there is no association between CP antibody titers and clinical presentation of coronary artery disease. We suggest that CP has limited effect on coronary atherosclerosis.* (Cardiol J 2008; 15: 245–251)

Key words: ***Chlamydophila pneumoniae*, atherosclerosis, gensini and extent score**

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Introduction

Atherosclerosis is a multifactorial process and it is considered to be an inflammatory disease [1, 2]. The inflammation plays a central role in both pathogenesis of atherosclerotic vascular disease and its atherothrombotic complications [3]. It has been postulated that chronic infection with various pathogens may promote arterial inflammation, thereby contributing to the initiation or progression of atherosclerosis. The potential role of infectious agents in the pathogenesis and progression of atherosclerosis has been studied in several studies recently [4–6].

Chlamydophila pneumoniae (CP), an important cause of atypical pneumonia is an obligate intracellular human pathogen [7]. Saikku et al. [8] showed that patients with coronary artery disease (CAD) had elevated antibody titers against CP. This has been detected in human atherosclerotic plaques [9]. Furthermore, recent clinical studies have investigated the possibility of treating CAD with anti-chlamydial antibiotic therapy [10–13].

Although present studies may differ, the role of CP on atherosclerosis is still a matter of controversy. Also no sufficient angiographic study is available about the impact of CP infection on severity and intensity of coronary atherosclerosis. Therefore in our study we aim to detect whether a relation exists between CP antibody titers and angiographic severity and intensity of coronary atherosclerosis and clinical presentation of CAD. In addition, we evaluated the highly sensitive C-reactive protein (CRP) levels and their relation both with CAD and CP infection.

Methods

Study population

The study population consisted of 516 patients (mean age: 56.8 ± 10.4 years, 326 M) in whom CAD was suspected and a coronary angiography was performed in the cardiology department of Erciyes University. The study was approved by the local ethics committee. All patients were informed and informed consent was obtained.

The patient group included 353 patients with angiographically proven CAD, which contained 3 different subgroups: 163 patients with myocardial infarction (MI), 106 patients with unstable angina pectoris (USAP) and 84 patients with stable angina pectoris (SAP). The control group included 163 subjects with angiographically proven normal coronary arteries.

The patients who had acute MI were defined by a positive troponin test and elevated cardiac markers with typical chest pain or electrocardiographic changes consistent with MI [6, 14]. Unstable angina was diagnosed if the patient had a positive or negative troponin test with normal cardiac markers or any one of the following criteria: new onset angina (< 2 months) of at least class III according to the Canadian Cardiovascular Society, prolonged (> 20 min) angina at rest, recent (< 2 months) worsening of angina pectoris, or angina that occurred within 2 weeks of an acute MI [15]. Stable angina pectoris was defined by its clinical presentation of discomfort in the chest, typically aggravated by exertion or emotional stress, and relieved by nitroglycerin administration or rest. Normal coronary artery was defined as a completely clean coronary artery without any obstructive or non-obstructive lesions.

The study was approved by the local bioethical committe and all patients gave their informed consent.

Exclusion criteria

Patients with active infective disease, known connective tissue diseases, pulmonary, renal, hepatic and hematological disorders were excluded from the study.

Blood analysis

Peripheral blood samples were taken from an antecubital vein after admission to the hospital. Serum fasting blood glucose, total cholesterol, triglyceride, and HDL-cholesterol levels were determined by enzymatic methods after 12 hours fasting. Lipid parameters were measured by the advice of Konelab 60I (Thermo Clinical Lab Systems) with original Thermo kits. LDL-cholesterol was calculated with Friedewald formula [16]. CRP was measured by Behring nephelometry system kits. A level above 6 mg/dl was accepted as a positive result.

Serological analysis

Blood samples were taken during a coronary angiography. Determination of specific IgG antibodies against CP were performed by ELISA enzyme immunoassay kit (CPG/0604) DIO.PRO as previously described [17–19]. The remaining blood was centrifuged for 10 to 30 minutes and separated. Plasma and serum specimens were frozen and stored at –70°C until analysis. In each instance, kits were stored at 4°C and allowed to stand at room temperature for one hour before use. The serum samples for analysis were diluted 1:101 with a sample buffer. In the first reaction step, diluted patient samples were incubated with the wells. To detect the

bound antibodies, a second incubation was carried out using enzyme-labelled anti-human IgG, which was capable of promoting a colour reaction. After washing and drying, photometric measurements of the colour intensity were made at a wave length of 450 nm and a reference wavelength of > 620 nm within 30 min of adding the stop solution. Results were expressed as enzyme immuno units (IU) calculated relative to a calibrator specimen. Seropositivity was defined as an IgG titer higher than cut off (5 IU/mL).

To evaluate the effect of low and high CP IgG antibody titers on coronary atherosclerosis, we classified patients into two subgroups as with low (< 50 IU/mL) and high (50 IU/mL) antibody titers. Coronary angiography findings were compared between these two groups.

Coronary angiography

Selective coronary angiography was performed with 6 F or 7 F Judkins catheters. All images were acquired on a Philips Integris H 5000 at 25 frames/ /second. The numbers of major vessels with any luminal stenosis (lumen diameter reduction) was scored from 1 to 3 vessel disease (right, left anterior descending, and circumflex arteries). Left main stenosis ≥ 50% was scored as two vessel disease if there was no lesion ≥ 50% in the other vessels. The results of the angiography were evaluated by two cardiologists who were blinded to the results for CP IgG levels.

Gensini score

We used the Gensini score to evaluate the severity of atherosclerosis. The modified Gensini score has been described and validated previously [20, 21]. The most severe stenosis in each of eight coronary segments was graded from 1 to 4 (1 to 49% lumen diameter reduction: 1 point, 50 to 74% stenosis: 2, 75 to 99% stenosis: 3, 100% occlusion: 4) to give a total score of between 0 and 32. This score therefore gives an index of the severity of coronary atherosclerosis.

Extent score

We used the extent score to evaluate the intensity of atherosclerosis. This score was developed by Sullivan et al. [22] to indicate the percentage (0% to 100%) of the coronary surface involved by atheroma. The proportion of each vessel involved by atheroma, as identified by lumen irregularity, was multiplied by a factor for each vessel: left main, 5; left anterior descending, 20; main diagonal branch, 10; first septal perforator, 5; left circumflex, obtuse marginal, and posterolateral vessels, 10; right coronary, 20; and main posterior descending branch, 10. When the major lateral wall branch was a large obtuse marginal or intermediate vessel, the factor used was 20, with factor of 10 for the left circumflex. Occluded vessels which were filled with contrast medium by collateral flow were evaluated according to the visible irregularities of the vessel wall. If no collateral flow existed, the mean value of all the other vessel segments in this angiogram were transferred to this occluded vessel segment. Therefore this score gives a measure for extent of coronary atherosclerosis

Statistical analysis

Data are expressed as mean ± SD, percentage or median (Interquartile (25^{th} to 75^{th}) ranges) (IQR). Kolmogorov-Smirnov tests were used to assess the distributions of numeric parameters. Spearman correlation analyses were performed to test for correlation analyses. Comparisons between the groups were carried out using Student's t test, Mann-Whitney U test, ANOVA, and the χ^2 test. All probability values reported are two-tailed, with values of $p < 0.05$ considered statistically significant. The SPSS 11.0 software was used for statistical analysis.

Results

Baseline characteristics are shown in Table 1. History of diabetes, hypertension, smoking, LDL level and age were similar in patient and control groups.

The mean IgG antibody titer was 44.3 ± ± 28.8 IU/mL in the patient group and 39.8 ± ± 27.4 IU/mL in the control group ($p = 0.14$) and the CP seropositivity ratio was similar between the two groups (patient group: 92.9% *vs*. control group: 91.7%, $p > 0.05$).

In the patient group, the number of diseased coronary arteries was 2.4 ± 0.9. The mean Gensini score was 6.7 ± 4.0 and the extent score was 27.2 ± ± 15.3. There was no statistically significant correlation between both the Gensini and extent score and CP IgG titers (with Gensini score: $r = +0.103$, $p = 0.07$, with extent score: $r = +0.110$, $p = 0.31$).

When we grouped the patients as high (> 50 IU/ /mL) or low (< 50 IU/mL) IgG antibody titers, IgG titers in 58% of all patients had above 50 IU/mL. The number of diseased coronary arteries was higher in patients with high IgG levels than patients with low IgG levels (2.6 ± 1.1 *vs*. 2.2 ± 0.8, $p = 0.01$, respectively). While Gensini score was significantly higher in patients with high IgG titers, extent

Table 1. Patient characteristics.

	Patient group (n = 353)	Control group (n = 163)	p
Age (years)	56.4 ± 10.1	57.2 ± 7.7	NS
Male (%)	62	47	< 0.05
Diabetes mellitus (%)	16.5	11.9	NS
Hypertension (%)	33.5	35.1	NS
Current smoking (%)	37	31	NS
Total cholesterol [mg /dL]	187 ± 39	184 ± 44	NS
LDL-cholesterol [mg /dL]	143 ± 36	144 ± 35	NS
HDL-cholesterol [mg /dL]	48 ± 12.	50 ± 21	NS
Triglycerides [mg/dL]	151. ± 105	157 ± 104	NS

Data are presented as the mean value ± SD or percentage of patients; p < 0.05 accepted statistically significant; NS — non significant

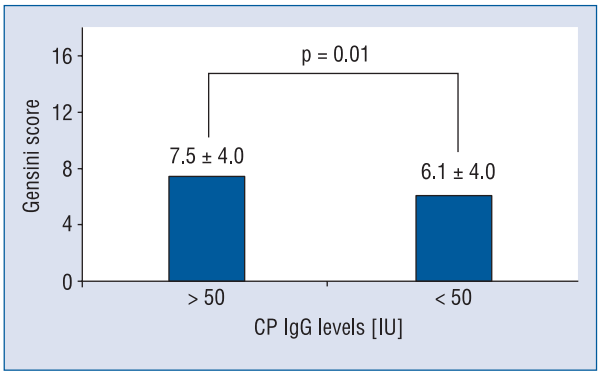

Figure 1. Gensini score in patients with a *Chlamydophila pneumoniae* (CP) IgG level above and below 50 IU/mL. Data are presented as the mean value ± SD; NS: p < 0.05 accepted statistically significant.

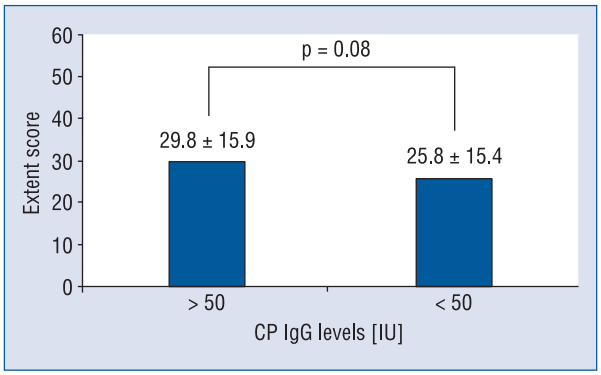

Figure 2. Extent score in patients with a *Chlamydophila pneumoniae* (CP) IgG level above and below 50 IU/mL. Data are presented as the mean value ± SD; NS: p < 0.05 accepted statistically significant.

Table 2. Comparison of IgG level and seropositivity in the subgroups.

	Myocardial infarction (n = 163)	Unstable angina pectoris (n = 106)	Stable angina pectoris (n = 84)	P
Rate of seropositivity (%)	95.5	91.1	92.9	0.7
IgG level [IU/mL] (X ± SD)	45.1 ± 28.6	45.2 ± 30.3	38.9 ± 28.6	0.4

Data are presented as the mean value ± SD or percentage of seropositivity

score did not change with IgG levels (Fig. 1, 2). In the subgroup analysis of patients with CAD, seropositivity ratios and IgG levels were similar between the three subgroups Table 2.

The CRP level was significantly higher in the patient group than the control group [median: 9.83 (IQR: 3–30) mg/dL *vs*. 3.3 (IQR: 3–10) mg/dL, respectively, p < 0.001]. The CRP level was positively correlated with both extent and Gensini score (r = +0.324, p < 0.001; r = +0.307, p < 0.001). However CRP levels in patients who had IgG level > 50 was similar to patients with low IgG levels [median: 15.2 (IQR: 4–46) *vs*. 11.0 (IQR: 3–30), respectively, p = 0.14].

The CRP levels were significantly higher in the MI group than the other two groups [median, MI: 20.9 (IQR: 7–59), USAP: 4.5 (IQR: 3–17), SAP: 3.6 (IQR: 3–10), p < 0.001] No significant correlation was observed between the CRP levels and the CP IgG titers (r =–0.031 p = 0.4).

Discussion

The association between CP infection and atherosclerosis is still not known. Despite a possible association that was shown in some studies [6, 8, 9, 23], there is limited data about the relation between CP infection and angiographic findings of coronary atherosclerosis. In some previous studies which investigated the association between CP infection and severity of atherosclerosis, atherosclerosis severity was evaluated by different methods in different patient groups. In one study, Imai et al. [24] evaluated the severity of atherosclerosis by the Gensini score but there were only patients with stable angina pectoris in this study. Wang et al. [25] investigated severity and extent of CAD by the number of major epicardial coronary arteries involved and by the Duke risk score. Although Yavuz et al. [26] investigated the relation between atherosclerosis and seropositivity in patients with suspected CAD, authors used only Gensini score for the severity of atherosclerosis. Moreover the relation between CP infection and the clinical presentation of coronary artery disease was not investigated in these studies. In our study, we evaluated atherosclerosis by diseased vessel number, Gensini and extent score. Therefore we aimed to determine whether CP infection increases the degree of atherosclerosis and affects the stages of atherosclerosis. We also investigated the relation between CP infection and CAD subgroup. According to our data, CP infection seems to be related only to angiographic coronary stenosis. Severity of atherosclerosis was higher in patients with high CP antibody titers. However, we did not find any relation between CP IgG levels and extent of atherosclerosis. Our results are concordant with a recently published study by Videm et al. [27]. The authors show that IgG against the CP protein antigen is more frequent in patients with significant coronary artery stenosis.

There are different opinions about which stage of atherosclerosis CP affects. Despite some studies suggesting that CP may affect early stages of atherosclerosis [28] other results do not support that idea [29, 30]. Gensini and extent scores which we used in our study may show different stages of atherosclerosis. While extent scores show the angiographic amount of plaque independent of stenosis severity, the Gensini score indicates the degree of stenotic coronary lesions which occur predominantly in the late phase of atherosclerosis. It is an acceptable result that the late phase of atherosclerosis may be evaluated by the Gensini score. According to our results, CP infection correlates only with stenosis severity not with the extent of atherosclerosis. These results show that CP infection may be associated with progression of the atherosclerotic process and this relation may become evident at high antibody levels. Ericson et al. [30], in a postmortem study, found that direct immunofluorescence for CP was positive in 86% of cases with severe atherosclerosis but in only 6% of cases with mild atherosclerosis. Although seropositivity for CP is rather high (70–80%) in the population, the fact that CP reactivity is very rare in mild lesions and very high in severe lesions may be histopathologic evidence of our opinion.

In the second part of the study, we demonstrated that although CP antibody titer was associated only with severity of stenosis, CRP level was associated with both severity and intensity of atherosclerosis. However we did not find any relation between CP infection and CRP levels. In a report, Anderson et al. [31] showed borderline significant association between elevated CRP and combined seropositivity for Helicobacter pylori and CP, but not for CP alone. Further, it was found there was no significant relation of CP seropositivity with plasma level of CRP in the Physicians Health Study [32]. In our study, obtaining no relation between CRP level and CP antibody titer or correlation between CRP levels and atherosclerosis make us consider that CRP is related to the atherosclerosis independent of CP infection and that chronic CP infection does not lead to the increase in systemic inflammatory activity such as CRP.

Another controversial subject is the relation between CP infection and the presentation of ACS. Our other purpose was to evaluate the role of CP infection in different clinical types of coronary artery disease, comparing serological data of patients between stable angina, unstable angina, and acute MI. Although previous studies failed to demonstrate an association between antibodies to CP and ACS [33–35], the association between CP infection and ACS was demonstrated in some studies. In a recent publication, Liu et al. [36] showed that the number of CP positive cells in coronary plaques was greater in ACS patients (21 patients) than in non-ACS patients (17 patients). However several clinical studies have investigated the possibility of treating coronary patients with antichlamydial antibiotic therapy [10–13]. In a meta analysis of the studies which investigated effects of anticlamydial therapy on the outcome of patients, there was no significant benefit on any of these end points such as total mortality,

MI, and ACS [37]. Despite the possible relation between CP infection and ACS, we found that the titers of antichlamydial antibodies and seropositivity of CP are not different in the subgroups of CAD. These findings indicate that although CP infection may affect progression of atherosclerosis, it does not play a significant role in inducing plaque activation or presentation of CAD. Both obtaining no reduction in cardiac event rate with antibiotic therapy for CP in large randomized controlled trials and serologic studies support our data.

Limitations of the study

The number of patients seronegative for CP infection was very low in our study. Therefore we could not evaluate severity and intensity of atherosclerosis between seropositive and seronegative patients. IgM antibody seropositivity for CP infection is very rare. We think that it would be difficult to find enough CP IgM positive patients to compare the groups therefore we only analyze IgG antibody titers. Although we evaluated severity and intensity of atherosclerosis, we did not investigate the relation between morphologic properties of atherosclerosis and CP infection. While we suggest that CP affects only the late phase of atherosclerosis, we evaluated atherosclerosis only angiographically. We think that for definitive evidence about which stage of atherosclerosis is affected by CP infection, intravascular ultrasound studies need to be performed.

Conclusions

In our study, we investigated the association between CP infection and coronary atherosclerosis and found that CP IgG antibody titers are associated with the severity of coronary stenosis at higher antibody levels. There is no association between CP antibody titers and the clinical presentation of CAD. We suggest that CP has limited effects on the progression of coronary atherosclerosis and these negative effects emerge only at high antibody levels.

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