Interrelationship of interleukin 6, C-reactive protein and *Chlamydia pneumoniae* IgG antibodies in patients with acute coronary syndromes

Međusobni odnos interleukina 6, C reaktivnog proteina i *Chlamidya pneumoniae* IgG antitela kod bolesnika sa akutnim koronarnim sindromom

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Abstract

**Background/Aim.** Inflammation due to infection could be associated with the development of acute coronary syndromes, clinical manifestations of ongoing atherosclerosis in vessel walls. Our aim was determine whether interleukin 6, C-reactive protein and *Chlamydia pneumoniae* IgG antibodies are connected with the development of acute coronary syndromes, to evaluate their interrelationship and to examine whether they are predictive of new events and mortality.

**Methods.** This prospective study included 211 subjects, of whom 111 were patients with acute coronary syndromes (60% male, mean age 59.42 years) and 100 were healthy controls (58% male, mean age 59.03 years). Blood samples were taken for analysis on admission, before the application of the therapy. Interleukin 6, high sensitivity C-reactive protein and *Chlamydia pneumoniae* IgG antibodies were measured, in a follow-up period of 30 days.

**Results.** Levels of interleukin 6 (p < 0.001) and C-reactive protein (p < 0.001) were significantly higher among the patients with acute coronary syndromes than among controls. Chronic infection caused by *Chlamydia pneumoniae* was present in 72% of patients and in 22% of healthy controls (p < 0.001). There was a correlation between interleukin 6 and C-reactive protein, C-reactive protein and *Chlamydia pneumoniae* but not between *Chlamydia pneumoniae* and interleukin 6. Higher levels of interleukin 6 and C-reactive protein were seen with increasing body mass index, smoking exposure, presence of hypertension and diabetes, and decreasing ejection fraction. The patients with ST-segment elevation had higher examined markers than the patients without ST-segment elevation. Interleukin 6 and C-reactive protein were independently related to the clinical outcome.

**Conclusion.** Interleukin 6, C-reactive protein and *Chlamydia pneumoniae* infection are connected with the development of acute coronary syndromes and may reflect a clinical outcome of the disease.

**Key words:** coronary disease; inflammation mediators; interleukin-6; C-reactive protein; chlamyphilia pneumoniae; prognosis.

Apstrakt

**Uvod/Gilj.** Inflamacija predstavlja patofiziološki mehanizam udužen sa nastankom akutnih koronarnih sindroma – kliničkih manifestacija uznemiredovalte arterioskleroze. Gilj ovog rada bio je ispitivanje povezanosti interleukina 6, C reaktivnog proteina i *Chlamydia pneumoniae* sa nastankom akutnih koronarnih sindroma, njihovog međusobnog odnosa i odnosa sa tradicionalnim faktorima rizika, kao i prediktivne uloge u nastanak novih događaja i letalnog ishoda. **Metode** Prospektivnom studijom bilo je obuhvaćeno 211 ispitanika: 111 bolesnika sa akutnim koronarnim sindromom i 100 zdravih osoba (kontrolna grupa). Uzorci krvi izviđeni su na prijemu, pre primene terapije. Određene su vrednosti interleukina 6, visoko osetljivog C reaktivnog proteina i titer antitela klase imunoglobulin G protiv *Chlamydia pneumoniae*. Period praćenja iznosio je 30 dana. **Rezultati.** Interleukin 6 (p < 0.001) i C reaktivni protein (p < 0.001) bili su značajno viši kod osoba oboljelih od akutnih koronarnih sindroma u odnosu na pripadnice kontrolne grupe. Hronična infekcija koja je uzrokovana *Chlamydiom pneumoniae* utvrđena je kod 77% bolesnika i 22% zdravih osoba (p < 0.001). Utvrđena je korelacija između interleukina 6 i C reaktivnog proteina, zatim C reaktivnog proteina i antitela protiv *Chlamydiae pneumoniae*. Porast vrednosti interleukina 6 i C reaktivnog proteina pratio je porast indeksa telesne mase, pušenje cigareta, prisustvo hipertenzije i dijabetesa, smanjenje ejekcije frakcije komora. Ispitivani markeri bili su značajno viši kod bolesnika sa elevacijom ST segmenta. Oba markera bila su prediktori novog događaja u periodu praćenja. **Zaključak.** Interleukin 6, C reaktivni protein i infekcija izažvana *Chlamydiom pneumoniae* povezani su sa nastankom akutnih koronarnih sindroma i mogu uticati na klinički ishod oboljenja.

**Key words:** korunarna bolest; zapaljenje, medijatori; interleukin-6; C reaktivni protein; chlamyphlia pneumoniae; prognoza.

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**Introduction**

Many molecular and cellular mechanisms link inflammation and haemostatic mechanisms in atherothrombosis, from the initiation to the clinical manifestation of the process.\(^1\)

Inflammation may promote thrombosis by acting both locally and systemically. It can affect systemic haemostatic activity by interleukin-6 (IL-6), the major cytokine responsible for the acute phase response. High sensitivity testing for C-reactive protein (CRP), a nonspecific marker of low grade inflammation, has received much attention. Several studies showed a strong link between elevation of CRP and the risk of future events. Other acute phase proteins, such as leucocytes count and various proteins concentration (fibrinogen, von Willebrand factor etc.) also have prognostic significance.\(^2\-^6\)

Recently, it has been hypothesized that various infective diseases, both bacterial and viral, may activate vessel associated leucocytes or immune reactions in arteriosclerotic process.

On the other hand, acute infections can alter haemodynamics and the clotting and fibrinolytic systems in ways that can precipitate ischemic events. Chronic extravascular infections can augment extravascular production of inflammatory cytokines that may accelerate the evolution of remote arteriosclerotic lesions. Intravascular infection might also provide local inflammatory stimuli that accelerate atherogenesis. Many human plaques show signs of infection by microbial agents such as *Chlamydia pneumoniae* (Cp) which can release heat shock proteins and stimulate the production of proinflammatory mediators by vascular endothelial and smooth muscle cells and infiltrating leucocytes.\(^7\-^9\)

All of the mentioned components involved in arteriosclerotic process are well studied in apparently healthy subjects.

The aim of the study was to investigate the relationship between levels of IL-6 and CRP and the development of acute coronary syndromes (ACS). Also, among the other widely investigated infectious agents, the aim was to determine the previous exposure to Cp infection in our patient population by measuring the titers of specific antibodies; possible interrelationship between examined markers, correlations with risk factors and clinical characteristics and their prognostic impact.

**Methods**

The prospective study included 211 participants of whom 111 were the patients admitted to Coronary Care Unit (CCU) of Clinic for Cardiovascular Diseases, Clinical Center Nis in Serbia between December 2002 and May 2004 due to acute coronary syndromes (ACS) documented by typical electrocardiographic (ECG) findings for myocardial ischemia (T-wave and ST-segment changes) and elevation of sensitive and specific biomarkers (troponin I and CK-MB).

Examination of patients included data about characteristic chest pain. The ECG findings were recorded (initial ST elevation, ST depression, T-wave inversion or nonspecific changes). Patients were monitored continuously in the CCU. All traditional risk factors for coronary artery disease (CAD) were marked: smoking, hypertension, dyslipidemia, diabetes, body mass index (BMI), family history of CAD. We noted previous myocardial infarction (MI) or previous coronary artery by pass grafting (CABG).

The patients required full medical therapy including various combinations of aspirin, intravenous nitrates, anticoagulants, angiotensin – converting enzyme inhibitors (ACEI), statins and/or β-blockers. Where needed, reperfusion was done by using the fibrinolytic agents. We evaluated the in hospital outcome and end-points in the follow-up period of 30 days. All the patients who entered the study were interviewed by phone. Follow-up information was obtained about cardiac death, MI or new onset of unstable angina.

Healthy volunteers, a total of 100 persons, composed the control group. The group was age and sex matched. The inclusion criteria for the control group were the absence of known coronary artery diseases (previous stable or unstable angina as well as previous myocardial infarction) or CAD. Initial ECG was recorded to confirm the absence of coronary artery disease. Volunteers were asked about the risk factors for CAD (smoking status, family history of CAD, hypertension, dyslipidemia, diabetes) and BMI was determined.

The exclusion criteria in the group of patients and also of controls were concomitant dilated cardiomyopathy, valvular heart disease, atrial fibrillation, major surgery or trauma within the previous months. All the patients and controls with known or suspected thrombotic disorders, systemic illness, autoimmune diseases, sepsis, alcohol liver diseases, chronic obstructive pulmonary diseases, acute respiratory infections, current infections, any etiology or infections within previous three weeks, malignancy and inflammatory diseases were also excluded.

The investigation conformed to the principles outlined in the Declaration of Helsinki. Signed informed consent or witnessed oral informed consent was obtained from all patients and healthy controls in accordance with the guidelines of the Ethical Review Committee of the School Medicine Nis, University of Nis and Clinical Center of Nis which had given a written approval of the study.

Blood was drawn from the patients with ACS immediately after admission, before the application of any therapy. Regular laboratory analyses were performed in our Central Laboratory by using standard methods (white blood cell – WBC counts: reference range 4.5 to 9.0 × 10^9/L and monocytes: reference range 0.0 – 0.8 × 10^9/L). For further analyses which were not possible to perform in Clinical Center in Nis, 10 ml of serum (patients and controls) were frozen and kept at −40 °C. Tubes were sent on dry ice with special permission of Ministry of Health of Serbia to Immunosciences Lab. Inc, Beverly Hills, California, USA where the analyses were performed between May and October 2004. We measured the levels of IL-6, high sensitive – hs CRP and Cp IgG antibodies.

Levels of IL-6 and hs CRP were measured using kits manufactured by Diagnostic Products Corporation, Los Angeles, CA on an IMMULITE Automated Immunoassay Analyzer. The IMMULITE system utilizes assay-specific, antibody or antigen coated plastic beads as the solid phase, alkaline phosphatase labeled reagent and a chemiluminescent substrate.
The IMMULITE system automates the entire assay process. Light emission was measured by a photomultiplier tube and the results were calculated for each sample using different calibrators and controls.

The established reference ranges of the lab performing the tests were from 0.7 to 4.6 pg/mL for IL-6, and from 0 to 1 mg/dL for hs CRP. The values higher than the established reference ranges were marked as positive.

Sera were tested at 1:200 dilution for Cp IgG antibodies by using an in-house enzyme-linked immunosorbent assay (ELISA).

Peptide LPTAVLNLTAWSLLNATALST from major outer membrane protein of Cp was synthesized by Biosynthesis, Louisville, TX, and used in this assay.

Enzyme-linked immunosorbent assay (ELISA) was used for testing antibodies against nine different specific antigens, peptides in the sera of the patients with atherothrombosis and control subjects. Antigens or peptides were dissolved in methanol at a concentration of 1.0 mg/ml, and then diluted 1:100 in 0.1 M carbonate bicarbonate buffer, pH 9.5, and 50 µl were added to each well of a polystyrene flat-bottom ELISA plate. Plates were incubated overnight at 4°C and then washed three times with 20 mM tris buffer saline (TBS) containing 0.05% Tween 20, pH 7.4. The nonspecific binding of immunoglobulin was prevented by adding a mixture of 1.5% bovine serum albumin (BSA) and 1.5% gelatin in TBS, and then by incubating for 2 h at room temperature, and then overnight at 4 °C. Plates were washed as in the above, and then serum samples diluted 1:200 in 1% BSA-TBS were added to duplicate wells and incubated for 2 h at room temperature. Sera from patients with Chlamydia infection with known high titers of IgG against different Cp antigens were used to rule out nonspecific antibody activities of inter- and intra-assay variability. Plates were washed, and then peroxidase-conjugated goat antihuman IgG antiserum (KPI, Gaithersburg, Maryland) diluted 1:400 in 1% BSA-TBS were added to duplicate wells and incubated for 2 h at room temperature. After washing five times with TBS-Tween buffer, the enzyme reaction was started by adding 100 µl of ophenylene diamine in citrate-phosphate buffer, pH 5.0 and hydrogen peroxide diluted 1:10,000. After 45 min, the reaction was stopped with 50 µl of 2 N H2SO4. The optical density (O.D.) was read at 492 nm by means of a microtitre reader. Several control wells containing all reagents, but human serum, were used for detecting nonspecific binding.

The curve was constructed by plotting the mean absorbance obtained from each calibrator against its concentration with absorbance on the vertical (y) axis, and concentration on the horizontal (x) axis. By using the mean absorbance value for each control and unknown samples corresponding concentration were determined. The test validation, calibrators and control sera were run for each test. The performance characteristics of this test were determined by using the serum samples; at 3 S.D. above the negative control, samples were considered positive.

The results of normally distributed continuous variables are expressed as the mean value ± standard deviation. Continuous variables with a nonnormal distribution are presented as median values (interquartile interval) and qualitative variables are presented as frequencies. Analysis of normality of the continuous variables was performed with the Kolmogorov–Smirnov test. Differences between the examined groups were assessed by unpaired t-test, Mann–Whitney U test and χ2 testing was used for discrete variables.

Correlations between continuous variables were analyzed with two-way Pearson correlation tests. Logistic regression was used to assess the univariate associations. Information regarding the development of combined end-point was available in all the patients included in the study. Binary logistic regression, backward stepwise selection, was used to derive final model of which significance levels of 0.01 and 0.05 were chosen to exclude and include terms, respectively.

The relationship between hs CRP levels and the end point was not of linear nature. Therefore, in order to fulfill the statistical requirement, hs CRP was logarithmically transformed before entering the multiple regression analysis. Differences were considered to be significantly important if the null hypothesis could be rejected with > 95% confidence. All p values were two-tailed. The SPSS 10.0 statistical software package was used for all calculations.

**Results**

Characteristics of 111 patients with ACS and 100 controls are shown in Table 1.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients (n=111)</th>
<th>Controls (n=100)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years), x±SD</td>
<td>59.42±5.4</td>
<td>59.03±3.56</td>
<td>0.63</td>
</tr>
<tr>
<td>Male sex [n(%)]</td>
<td>67 (60.3)</td>
<td>58 (58)</td>
<td>0.72</td>
</tr>
<tr>
<td>Current or ex- smokers, [n(%)]</td>
<td>72 (64.8)</td>
<td>60 (60)</td>
<td>0.46</td>
</tr>
<tr>
<td>Dyslipidemia (%)</td>
<td>47 (42.3)</td>
<td>3 (3)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>67 (60.3)</td>
<td>30 (30)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>29 (26.1)</td>
<td>0 (0)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Family history of Coronary artery diseases (%)</td>
<td>67 (60.3)</td>
<td>30 (30)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Body masa index (kg/m²), x±SD</td>
<td>26.98±2.01</td>
<td>24.88 (1.48)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L), x±SD</td>
<td>6.03±1.34</td>
<td>4.82 (0.50)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Triglycerides (mmol/L), x±SD</td>
<td>2.26±1.53</td>
<td>1.35 (0.3)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>White blood cell count (x10⁹/L), x±SD</td>
<td>10.76±2.48</td>
<td>6.68 (1.04)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Monocytes (x10⁹/ L), median (range)</td>
<td>0.9 (0.7–1.22)</td>
<td>0.5 (0.3–0.7)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

The majority of the study participants were male (60.3% of the patients). The mean age of the patients was 59.42 and of controls 59.03 ($p = 0.63$). A high proportion of patients, but also of controls were smokers ($p = 0.46$). We found elevated white blood cells count (WBC) in patients with ACS ($p < 0.001$). Also, monocytes were significantly higher in patients than in the controls ($p < 0.001$).

Table 2 outlines clinical characteristics of patients with ACS. Out of 111 patients involved in the study, the presenting ECG showed ST – segment elevation in 50 patients. The mean value of systolic blood pressure was 132±35.98 mmHg. The patients spent in the hospital a period of 10.8±5.4 days to fulfill medical treatment. Previous MI was recorded in 33 patients and previous CABG in 19 (17.1%). Aspirin had been used before hospital admission by 38 (34.2%) patients. Fibrinolytic therapy (chiefly streptokinase) had been received by 44 (39.6%) patients. During the hospital stay 106 patients received aspirin and 106 received anticoagulant therapy (chiefly low molecular weight heparin). Intravenous nitrates had been received by 105 patients, β-blockers by 97, ACEI by 72 and statins by 60 of them. Five patients had persistent sinus bradycardia, which required atrial pacing. In our group of patients echo was performed on 89.2% of the patients.

Table 2: Clinical characteristics of patients with acute coronary syndromes (ACS)

<table>
<thead>
<tr>
<th>Characteristics of patients with ACS (n = 111)</th>
<th>μ±SD</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrocardiography abnormalities at entry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST-segment elevation</td>
<td>50 (45)</td>
<td></td>
</tr>
<tr>
<td>without ST-segment elevation</td>
<td>61 (55)</td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (BP) (mmHg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 120</td>
<td>36 (32.4)</td>
<td></td>
</tr>
<tr>
<td>120–139</td>
<td>24 (21.6)</td>
<td></td>
</tr>
<tr>
<td>140–159</td>
<td>17 (15.4)</td>
<td></td>
</tr>
<tr>
<td>≥ 160</td>
<td>34 (30.6)</td>
<td></td>
</tr>
<tr>
<td>Mean systolic BP (mmHg)</td>
<td>132±35.98</td>
<td></td>
</tr>
<tr>
<td>Mean diastolic BP (mmHg)</td>
<td>79±23.33</td>
<td></td>
</tr>
<tr>
<td>Heart rate (heartbeats/min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 70</td>
<td>18 (16.3)</td>
<td></td>
</tr>
<tr>
<td>70–89</td>
<td>43 (38.7)</td>
<td></td>
</tr>
<tr>
<td>90–109</td>
<td>41 (36.9)</td>
<td></td>
</tr>
<tr>
<td>≥ 110</td>
<td>9 (8.1)</td>
<td></td>
</tr>
<tr>
<td>Mean heart rate (heartbeats/min)</td>
<td>85±22.73</td>
<td></td>
</tr>
<tr>
<td>Previous disease and drug use</td>
<td></td>
<td></td>
</tr>
<tr>
<td>previous myocardial infarction</td>
<td>33 (29.7)</td>
<td></td>
</tr>
<tr>
<td>previous coronary artery by pass grafting</td>
<td>19 (17.1)</td>
<td></td>
</tr>
<tr>
<td>aspirin before admission</td>
<td>38 (34.2)</td>
<td></td>
</tr>
<tr>
<td>Duration of staying in hospital (days)</td>
<td>10.8±5.4</td>
<td></td>
</tr>
<tr>
<td>Treatment during hospital stay</td>
<td></td>
<td></td>
</tr>
<tr>
<td>fibrinolytic agents</td>
<td>44 (39.6)</td>
<td></td>
</tr>
<tr>
<td>aspirin</td>
<td>106 (95.4)</td>
<td></td>
</tr>
<tr>
<td>ticlopidin</td>
<td>7 (6.3)</td>
<td></td>
</tr>
<tr>
<td>anticoagulant</td>
<td>106 (95.4)</td>
<td></td>
</tr>
<tr>
<td>β blocker</td>
<td>97 (87.3)</td>
<td></td>
</tr>
<tr>
<td>angiotensin converting enzyme inhibitors</td>
<td>72 (64.8)</td>
<td></td>
</tr>
<tr>
<td>nitrate (intravenous)</td>
<td>105 (94.5)</td>
<td></td>
</tr>
<tr>
<td>antiarrhythmics</td>
<td>32 (28.8)</td>
<td></td>
</tr>
<tr>
<td>statins</td>
<td>60 (54)</td>
<td></td>
</tr>
<tr>
<td>Left ventricle ejection fraction (%)</td>
<td>54.4±13.30</td>
<td></td>
</tr>
<tr>
<td>Left ventricle ejection fraction &lt; 40</td>
<td>16 (14.4)</td>
<td></td>
</tr>
<tr>
<td>New event</td>
<td>44 (39.6)</td>
<td></td>
</tr>
<tr>
<td>Death, any cause</td>
<td>13 (11.7)</td>
<td></td>
</tr>
<tr>
<td>arrhythmia</td>
<td>4 (3.6)</td>
<td></td>
</tr>
<tr>
<td>cardiac rupture</td>
<td>1 (0.9)</td>
<td></td>
</tr>
<tr>
<td>cardiogenic shock</td>
<td>5 (4.5)</td>
<td></td>
</tr>
<tr>
<td>reinfarction</td>
<td>3 (2.7)</td>
<td></td>
</tr>
</tbody>
</table>

10.8±5.4 days to fulfill medical treatment. Previous MI was recorded in 33 patients and previous CABG in 19 (17.1%). Aspirin had been used before hospital admission by 38 (34.2%) patients. Fibrinolytic therapy (chiefly streptokinase) have been used by 44 (39.6%) patients. During the hospital stay 106 patients received aspirin and 106 received anticoagulant therapy (chiefly low molecular weight heparin). Intravenous nitrates had been received by 105 patients, β-blockers by 97, ACEI by 72 and statins by 60 of them. Five patients had persistent sinus bradycardia, which required atrial pacing. In our group of patients echo was performed on 89.2% of the patients.

During follow-up, 44 patients had a new event (death, new MI: reinfarction or MI after unstable angina, or new episodes of UA). For the primary composite outcome of death, 13 (11.7%) patients died during the first 48 hours in the hospital. Causes of death are shown in Table 2.

Our study showed that 46% of the patients had detectable levels of circulating IL-6 (Figure 1). This proportion is statistically significantly higher as compared to the controls (only 2% positive samples), $\chi^2$ test, $p < 0.001$; odds ratio (OR), 41.57.
Concentrations of IL-6 were significantly higher in patients compared to controls (4.7 pg/mL (4.00 – 8.85) vs 1.5 pg/mL (1.2 – 1.8), \( p < 0.001 \)) (Figure 2-A).

There was a significant difference between the patients and the controls regarding to CRP (Figure 1); 51% of the patients had CRP above the referent range as compared to 8% of controls (\( \chi^2 \) test, \( p < 0.001 \); OR, 11.97; 95% CI, 4.97 – 29.81).

The median of this marker of inflammation was 1.2 mg/dL (0.384 – 2.895) in the patients and 0.225 mg/dL (0.075 – 0.623) in controls (Figure 2-B), (Mann-Whitney U test, \( p < 0.001 \)).

According to our results, there was a significant difference between the patients and the controls in regard to the presence of Cp IgG antibodies: 72% of the patients were positive versus 22% of the controls, (\( \chi^2 \) test \( p < 0.001 \); OR, 9.12; 95% CI, 4.57 – 18.35) (Figure 1). IgG titers were significantly higher in patients compared to the controls: 0.825 o.d. (0.669 – 1.00) vs 0.552 o.d. (0.410 – 0.705), (\( t \)-test, \( p < 0.001 \)) (Figure 2-C).

In an effort to examine, the inflammatory immune reactions concentrations of IL-6 and CRP were analyzed. Pearson’s linear correlation showed a correlation between IL-6 and CRP (\( r = 0.454; p = 0.01 \)).

Our data also showed a correlation between concentration of CRP and circulating Cp IgG antibodies, \( p = 0.01 \). We did not find any correlation between circulating levels of IL-6 and Cp IgG antibodies (Pearson’s linear correlation, \( p = 356 \)).

Concentrations of IL-6 were higher in women (\( p = 0.03 \)), patients with previous MI (\( p = 0.03 \)), previous CABG (\( p = 0.03 \)), smokers (\( p = 0.001 \)), patients with hypertension (\( p = 0.001 \)) and with diabetes (\( p = 0.01 \)) and correlated with BMI (\( p = 0.01 \)). Significant correlations were not found between IL-6 levels and age (\( r = 0.185, p = 0.081 \)), triglyceride levels (\( p = 0.09 \)), total cholesterol levels (\( p = 0.08 \)), monocytes and WBC count (\( r = 0.008, p = 0.947 \)), (Table 3).

In similar fashion, CRP was also higher in women (\( p = 0.03 \)), patients with previous history of MI (\( p = 0.03 \)), previous CABG (\( p = 0.03 \)), smokers (\( p = 0.001 \)), hypertensive patients (\( p = 0.01 \)) and patients with diabetes (\( p = 0.05 \)). Positive and significant relationships were demonstrated between CRP levels and total cholesterol serum levels (\( r = 0.333, p = 0.01 \)), with monocytes count (\( p = 0.01 \)) and BMI (\( p = 0.01 \)). Interestingly, CRP levels were not significantly associated with serum triglyceride levels (\( p = 0.085 \)), with WBC count and age (\( r = 0.156, p = 0.134 \)) (Table 3).

Increasing WBC count was positively associated with increasing titers of Cp IgG antibodies (\( r = 0.252, p = 0.05 \)). Even stronger association was noticed between the increasing number of monocytes and increasing titers of Cp IgG antibodies (\( r = 0.356, p = 0.01 \)). Titers of Cp antibodies were higher in smokers than in non-smokers (\( p = 0.001 \)) (Table 4).

In an effort to examine the relationship between ECG findings on admission and vascular inflammatory markers, concentrations of IL-6 and CRP were analyzed in the subgroups of patients with or without ST-segment elevation. There was a significant correlation regarding to severity of the diseases. The patients with acute MI with ST-segment
Elevation had higher circulating levels of IL-6 \((p = 0.03)\) and CRP \((p = 0.05)\). The high-risk ECG was followed by higher concentrations of inflammatory markers.

Echocardiography was not essential early after myocardial infarction, but was useful after MI to assess left ventricular function. Left ventricle ejection fraction (LVEF) was an important prognostic variable in the patients with ACS.

Both, IL-6 levels and CRP levels showed a strong and significant correlation with LVEF (Pearson’s linear correlation, \(r = -0.359, p = 0.01\) for IL-6 and \(r = -0.371, p = 0.01\) for CRP) (Figure 3 and 4). However, there was no correlation between Cp IgG antibodies titer and LVEF \((r = -0.059, p = 0.619)\).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>IL-6</th>
<th>CRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.081</td>
<td>0.134</td>
</tr>
<tr>
<td>Women</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.001</td>
<td>0.01</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.01</td>
<td>0.05</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.08</td>
<td>0.01</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.09</td>
<td>0.085</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Current smoker</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Prior myocardial infarction</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>Prior coronary artery by pass grafting</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>White blood cell count</td>
<td>0.947</td>
<td>0.134</td>
</tr>
<tr>
<td>Monocytes</td>
<td>0.964</td>
<td>0.01</td>
</tr>
<tr>
<td>ST-elevation</td>
<td>0.03</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Finally, to examine independent correlates of IL-6, CRP and Cp IgG antibodies in prediction of a new event or death in a follow-up period, adjustment analyzes were performed by using binary logistic regression. IL-6 \((p = 0.007)\) and WBC count \((p < 0.001)\) were strong independent factors of death (Table 5). Kolmogorov Smirnov test previously showed abnormal distribution of CRP. Logarithmically normalized CRP showed positive but weaker correlation \((p = 0.035)\) as an independent predictor of death.

Similarly, IL-6 was independently associated with new coronary event \((p = 0.013)\), as well as age \((p < 0.001)\) (Table 6). There were no associations between CRP and new or recurrent MI or rehospitalization for ACS in 30-day of the follow-up.
Discussion

The aims of this study were to determine the possible relationship between IL-6, CRP and Cp IgG antibodies in patients presented with ACS-clinical manifestation of the latter phase of atherothrombosis.

To our knowledge, these are the first reports of the previous exposure to Cp pathogen and of an association with inflammatory-immune markers in our patient population with ACS.

The results suggest that the high proportion of patients had high titers of Cp IgG, high circulatory levels of both IL-6 and CRP, indicated the ongoing inflammation in ACS.

Our understanding of the pathogenesis of the acute thrombotic complications of the arteriosclerosis has widen in recent years. We now understand that many acute thrombotic coronary occlusions do not necessarily result from critically stenosed sites in the arteries. This distinction between lesions versus lumen diameter challenges our traditional reliance upon coronary anatomy. As in the initiation and progression of arteriosclerosis, ample data support the involvement of inflammation in these thrombotic complications of arteriosclerosis 10-13. The inflammatory stimuli produced by primary proinflammatory cytokines may undergo amplification through the induction of IL-6 production. Multiple cell types including vascular smooth muscle cells, endothelial cells, adipocytes can produce large amounts of IL-6.

A soluble mediator, IL-6, is a primary stimulant for the hepatic acute phase response and the only cytokine capable of inducing all acute phase proteins involved in the inflammatory response 14. As such, the induction of CRP by IL-6 underline atherogenesis. Similar to this finding, our data confirmed the correlation between CRP and IL-6.

C-reactive protein, an acute phase reactant and important component of the innate immune system, has emerged as not only a strong biomarker of vascular disease, but also as a potential participant in it. Multiple prospective epidemiological studies are focused on CRP that has been shown to predict incident myocardial infarction, stroke and peripheral arterial diseases. These highly consistent data are supported by laboratory evidence demonstrating that atherothrombosis, in addition to being a disease of lipid accumulation, represents a chronic inflammatory process 15-18.

If we have a proof of ongoing inflammatory response in the wounded coronary arteries there remains the question of a trigger that might initiate and sustain the process. Among “candidates” that could trigger both inflammatory and autoimmune responses, there is infection 19. Since the original studies of Saikku et al. 20 published in 1998, Cp bacteria have been considered the main vascular pathogens increasing the risk for CAD. Meta-analysis performed by Danesh et al. 21 in 2000 identified 15 prospective studies of Cp and CAD. In our study Cp antibody titers were also elevated. In the present patient population, the proportion of subjects with a high antibody response against Cp was 72%. The findings of Arnholm et al. 22 are similar. The observed antibody responses for microbes in our study may reflect chronic, but also reactivated latent infections. The findings of Kark et al. 23 and Sheehan et al. 24 do not support an association between specific antigen and ACS.

Chlamydia pneumoniae is responsible for a variety of respiratory illnesses and can be distributed via monocytes from respiratory tract to distant sites in the organism 25. Our study confirmed a correlation between monocytes and Cp antibodies. In the same fashion there was a correlation between WBC and Cp antibodies. If an infected macrophage attaches to the vessel wall, it may infect the cells lining the arterial surface and the artery would then attract more immune cells. And, the vicious circle is triggered.

The host response to infectious agents usually involves a change in the program of hepatic protein synthesis. The cytokine IL-6 may mediate much of this switch from pro-

| Table 5 | Multivariate predictors (Logistic regression) of death in 111 patients with acute coronary syndromes |
|-----------------|-----------------|-----------------|-----------------|
| Interleukin-6   | 1.084           | 0.007           | 1.023           | 1.149           |
| White blood cells | 0.629           | 0.000           | 0.488           | 0.809           |
| Log hs CRP*     | 2.456           | 0.035           | 1.065           | 5.661           |

*Log hs CRP, logarithmically transformed high sensitivity C-reactive protein. Variables entered in step 1: Chlamydia pneumoniae IgG antibodies, smoking status (including current and ex-smokers, years of age, monocytes count, diabetes, previous myocardial infarction, gender.

| Table 6 | Multivariate predictors (Logistic regression) of new event in patients with acute coronary syndromes |
|-----------------|-----------------|-----------------|-----------------|
| Interleukin-6   | 1.059           | 0.013           | 1.012           | 1.108           |
| Years of age    | 0.984           | 0.001           | 0.974           | 0.993           |

Variables entered in step 1: Chlamydia pneumoniae IgG antibodies, smoking status (including current and ex-smokers), years of age, monocytes count, diabetes, previous myocardial infarction, gender.
duction of ‘housekeeping proteins’ (such as albumin), to
greater synthesis of acute phase reactants. Our study demo-
strated a correlation between circulating titers and concen-
tration of CRP, indicating that degree of infection is followed
by inflammatory-immune response.

Major traditional risk factors of cardiovascular diseases
are smoking and diabetes and hypertension, obesity and dys-
lipidemia. We found that cigarette smoking may be rel-
ated to elevated levels of IL-6, CRP and Cp IgG antibodies.
The mechanism whereby smoking is related to concentra-
tions of IL-6, CRP and Cp is multifactorial and can be due to
bronchial injury, endothelial activation and systemic in-
flammation, as described elsewhere 26.

We demonstrated that several other risk factors to be rel-
ated to levels of IL-6 and CRP - specifically, the presence of
hypertension, diabetes and BMI. Nowadays, this cluster of
cardiometabolic risk factors is known as metabolic syndrome
– a condition underlined by low grade systemic inflammation,
or condition with systemic proinflammatory burden 27,28.

Inflammation tightly regulates the procoagulant poten-
tial of the arteriosclerotic plaque. Thus, in the setting of local
inflammation in the plaque or systemic inflammation as re-
lected by the acute-phase response, inflammatory pathways
conspire to promote thrombosis and combat fibrinolysis.
Consequently, inflammation promotes clot formation and in-
stability and underlines the thrombotic aspects of athero-
thrombosis, as well as the lesion initiation and progression 29.
If the thrombi are large or occlusive they lead to the acute
coronary syndromes.

Our results showed that IL-6 and CRP concentrations
are higher in the patients presented with ACS with ST-
segment elevation compared to patients presented with ACS
without ST-segment elevation. Moreover, we found that
both, IL-6 and CRP concentration correlate with LVEF. Such
results suggest that the levels of inflammatory markers cor-
relate with the severity of a disease.

Importantly, IL - 6 and CRP concentrations, but not Cp
antibodies, predict future death in the group of patients with
ACS (confirmed by using multiple linear models). These
findings indicate that levels of CRP, IL - 6 and WBC are
markers of disease activity. In the same fashion, IL - 6 was
the best predictor of new cardiovascular event, manifested as
new MI or UA.

The finding that an elevated WBC count was associated
with a higher mortality rate is in agreement with previous
studies 30,31. The basis for this remains unknown. It is pos-
ible that WBC count is a cause or simply a consequence of
ACS and that the degree of elevation of WBC count reflects
the severity of a disease.

Conclusion

Our study indicated that IL-6, CRP and Cp antibodies
were connected with the development of ACS, severity of
diseases and might reflect the clinical outcome in our patient
population. Interleukin-6, CRP and Cp antibodies were also
associated with several traditional risk factors, supporting the
idea of separate or concurrent pathway towards atherothrom-
bose and indicating the possibility of direct vascular effect
on plaque stability. Future studies should find out the exact
evidences of causes or consequences of arteriosclerotic coro-
nary artery disease.

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