Prevalence of *Chlamydia Pneumoniae* and *Mycoplasma Pneumoniae* in Different Forms of Coronary Disease

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Summary

Background: **Several infectious agents have been investigated since the association between atherosclerosis and infection was demonstrated; however, the results of these studies are contradictory.**

Objective: **To test the association between serum titers of anti-*Chlamydia* and anti-*Mycoplasma* antibodies in different forms of acute coronary syndromes (ACS).**

Methods: **One hundred and twenty-six patients were divided in 4 groups: ACS with ST- segment elevation (32 patients), ACS without ST-segment elevation (30 patients), chronic coronary artery disease (30 patients) and blood donors without known coronary disease (34 patients - control group). In the two first groups, serum samples were collected at hospital admission (first 24 hours of hospitalization) and after a 6-month follow-up. In the other two groups, only a basal sample was collected. Anti-*Chlamydia* and anti-*Mycoplasma* antibodies were measured by indirect immunofluorescence in all samples.**

Results: **Significant differences were observed between the basal sample and the one measured after a 6-month follow-up in patients with myocardial infarction with ST-segment elevation for *Chlamydia* (650±115.7 versus 307±47.5, p=0.0001) as well as *Mycoplasma* (36.5±5.0 versus 21.5±3.5, p=0.0004). The groups with ACS had higher anti-*Chlamydia* and anti-*Mycoplasma* serum antibody levels in the basal measurement, when compared to the patients with chronic coronary disease and the control group, but the differences were not statistically significant.**

Conclusion: **The present study showed an association between the serum titers of anti-*Chlamydia* and anti-*Mycoplasma* antibodies in the acute phase of patients with unstable angina or myocardial infarction. (ClinicalTrials.gov - NCT00561028). (Arq Bras Cardiol 2009; 92(6) : 405-411)**

Key words: ***Chlamydophila pneumoniae; mycoplasma pneumoniae*; atherosclerosis; coronary artery disease.**

Introduction

Since 1978, when the association between infection and atherosclerosis was first demonstrated[1], several infectious agents have been investigated[2]. Among these, *Chlamydia pneumoniae* (Cp) is the one that presents higher evidence of its participation in stable atherosclerotic disease, acute myocardial infarction (AMI) and cerebrovascular accident (CVA)[3-7].

However, the contradictory results of the studies that tested the effects of anti-Cp antibiotics on the decrease of cardiovascular events, raised some doubts about the participation of the infection and, more specifically, of Cp in atherosclerotic disease.

Higuchi et al[8,9], in a study of necropsy specimens in 2000, confirmed a high number of cells infected by Cp in atherosclerotic plaques, also demonstrating for the first time the presence of another infectious agent in the site, later identified as *Mycoplasma pneumoniae* (Mp)[8,9].

This infectious agent behaves as super-antigen and needs cholesterol to survive, as its membrane is comprised of this substance[10]. From the physiopathological point of view, an interesting hypothesis is that the Mp would work as "trigger" to activate the Cp, thus promoting the destabilization of the coronary atherosclerotic plaque.

This pilot study was planned to evaluate, through a serological investigation, the role of these two bacterial agents in the triggering of acute coronary ischemic events.

Methods

Study design

This study was approved by the local Ethics Committee in Research and all patients signed the free and informed consent form prior to study enrollment.

From March 2002 to November 2004, a total of 138 patients were prospectively included, as follows:

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a) 34 patients with AMI and ST-segment elevation (mean age 55.1±1.7 years, with 73.5% of the male sex);

b) 40 patients with unstable angina or AMI without ST-segment elevation (mean age 60.1±2.1 years, with 52.5% of the male sex);

c) 30 patients with chronic atherosclerosis, asymptomatic, or with stable angina (mean age 65.9±2.0 years, with 63.3% of the male sex);

d) 34 individuals without known coronary disease who were blood donors, (mean age 42.5±1.2 years, with 64.7% of the male sex).

Primary objective

The main objective of study was to test the association between serological titers of anti-*Chlamydia pneumoniae* and anti-*Mycoplasma pneumoniae* (Anti-Cp e Anti-Mp) antibodies and Acute Coronary Syndrome (ACS).

Inclusion criteria

Patients of both sexes, aged 18 and older, were included in the study after signing the informed consent form. The different groups were thus defined:

a) ACS with ST-Segment Elevation

This group consisted of patients that presented pain with characteristics of angina ≤ 24 hours of evolution that met at least two of the following AMI criteria:

• *Clinical:* high-intensity and oppressive retrosternal pain, lasting longer than 20 minutes, which did not respond to nitrates;

• *Electrocardiographic:* ST-segment elevation ≥ 1mm in at least two peripheral derivations and/or ST-segment elevation ≥ 2 mm in at least two consecutive precordial derivations and/or presence of left branch block.

• *Myocardial necrosis marker:* at least two-fold increase in relation to normal values of CKMB activity (normal up to 25U/l) and/or Troponin T (normal up to 0.010 ng/ml).

b) ACS without ST-Segment Elevation

This group consisted of patients that presented pain with characteristics of angina ≤ 24 hours of evolution with (AMI without ST-Segment Elevation) or without (unstable angina) increase in biomarkers of myocardial necrosis (CKMB and/or troponin T.

Only high-risk patients were selected from this group, according to the classification of the Brazilian Society of Cardiology[11].

• Age > 75 years

• Progressive pain, symptoms in the last 48 hrs;

• Prolonged pain (> 20 min.), at rest;

• Pulmonary edema, worsening or onset of mitral regurgitation murmur, B3, hypotension, bradycardia and tachycardia.

• ST segment depression ≥ 0.5 mm (associated or not with angina), dynamic ST segment alteration, complete branch block, new or presumably new. Sustained ventricular tachycardia

• Extremely elevated ischemia markers (>99th percentile)

c) Chronic atherosclerosis group

Patients with chronic coronary atherosclerotic disease or with stable angina. The presence of the disease was defined by the following criteria:

• Previous AMI and/or;

• Previous surgical myocardial revascularization and/or;

• Previous percutaneous coronary intervention and/or;

• Previous coronary angiography demonstrating obstructive lesions of coronary arteries.

d) Control group

Individuals randomly selected from blood donors at Hospital de Base, who denied the presence of any known atherosclerotic disease.

Exclusion criteria

Patients that presented at least one of the following criteria were excluded:

• Difficulty to understand the informed consent form;

• Refusal to sign the informed consent form;

• Impossibility to return for the study follow-up;

• Age younger than 18 years;

• Time of evolution > 24 hours;

• Active infection;

• Use of antimicrobial agents on the last 30 days;

• Terminal diseases.

Blood collection methodology

In the groups with ACS with and without ST-segment elevation, blood samples were collected at two occasions, the first during the acute event and the second six months later. In the other two groups (chronic atherosclerosis and control groups), these samples were collected only once. As the half-life of the IgG antibodies is 30 days, the collection six months after the acute event aimed at reflecting the basal values of an individual chronically infected by Cp as well as by Mp.

The IgG anti-Cp and anti-Mp antibodies were measured in all samples and the results were expressed by the highest dilution value in which the reaction was positive.

Laboratory assessment

The serological tests were performed by a single, blinded and experienced examiner, using the indirect immunofluorescence technique[12].

Statistical analysis

The categorical data are presented as absolute numbers and percentages ad the continuous variables as means ± standard error (SE) for clarity. The continuous variables did not have a Gaussian distribution and were assessed by non-parametric tests (Kruskal-Wallis and Wilcoxon)[13]. Box plot

charts are presented in medians and interquartiles; *p* values < 0.05 were considered statistically significant (two-tailed). The software used for the statistical analysis was StatsDirect Statistics Software, v. 2.6.5.

Results

The recommended sample size consisted of 30 patients per group, totaling 120 patients.

Eight patients died during the study: 2 from the ACS group with ST elevation and six from the ACS group without ST elevation. Four patients from the ACS without ST elevation did not attend the pre-scheduled follow-up appointments and, after several unsuccessful attempts to contact them, they were considered as lost to follow-up and, therefore, it was not possible to collect the second blood sample in these cases.

These losses were replaced and a total of 138 patients were included in the study: 34 in the ACS group with ST elevation; 40 patients in the ACS group without ST elevation; 30 patients in the chronic atherosclerosis group and 34 normal individuals in the control group. Only patients that were submitted to all planned serologic assessments for the respective group were included in all the study analyses.

Clinical characteristics of the patients

Table 1 shows the clinical characteristics of the 126 patients that underwent all planned serologic assessments. It is worth mentioning the high prevalence of risk factors found in the chronic atherosclerosis group 83.3% were hypertensive, 53.3% were smokers and 90% presented dyslipidemia).

Comparison between the serologic analyses obtained at admission and 6 months after hospital admission in the groups with and without ST-segment elevation

Charts 1 and 2 demonstrate the serologic values obtained at the acute phase of the coronary event and six months after it, in the groups with and without ST-segment elevation. This analysis was the main objective of the study.

The patients with ACS and ST-elevation presented a significant decrease in the serologic titers from the samples obtained six months after hospital admission, regarding the Cp (p=0.0001) as well as the Mp (p=0.0004).

The same serologic behavior was not observed in the group without ST elevation, for Cp as well as for Mp (p=0.27 and 0.99 respectively).

Comparison between the serologic values obtained at the start of the study

Charts 3 and 4 compare the serologic differences among the different studied groups (first serologic sample). It can be observed that, in the groups that presented acute myocardial ischemia (ACS with and without ST elevation), higher serologic values were observed when compared to the groups that did not have the acute event (chronic atherosclerosis and control), regarding Cp as well as Mp, although these differences were not statistically significant.

Discussion

The present study was motivated by the need to clinically evaluate the concomitant presence of *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* in coronary

Table 1 – Clinical characteristics of the population (126 patients)

Clinical characteristics n (%)	ACS with ST-segment elevation (n = 32)	ACS w/t ST-segment elevation (n = 30)	Stable CAD (n = 30)	Control (n = 34)	p value
Age (mean ± SE)	55.1 ± 1.7	60.1 ± 2.1	65.9 ± 2.0	42.5 ± 1.2	< 0.0001
Men	24 (75)	17 (56.7)	19 (63.3)	22 (64.7)	0.5007
History of hypertension	15 (46.9)	22 (73.3)	25 (83.3)	N/A	0.0065
History of diabetes	4 (12.5)	6 (20.0)	5 (16.7)	N/A	0.7252
Smoking	22 (68.7)	11 (36.7)	16 (53.3)	N/A	0.0407
Previous angina	15 (46.9)	21 (70.0)	8 (26.7)	N/A	0.0035
Previous AMI	7 (21.9)	10 (33.3)	20 (66.7)	N/A	0.001
Family history of CAD	14 (43.7)	13 (43.3)	17 (56.7)	N/A	0.4977
Dyslipidemia	12 (37.5)	10 (33.3)	27 (90.9)	N/A	<0.0001
Previous statin use	4 (12.50	3 (10.0)	24 (80.8)	N/A	< 0.0001
Previous PCI	3 (9.4)	3 (10.0)	8 (26.7)	N/A	0.104
Previous MR	2 (6.2)	3 (10.0)	14 (46.7)	N/A	< 0.0001

W/t- without; ACS – acute coronary syndrome; CAD – coronary artery disease; SE – standard error; AMI – acute myocardial infarction; PCI – percutaneous coronary intervention; MR – myocardial revascularization.

atheromatous lesions, previously described by Higuchi et al[9] and cols. in necropsy findings.

Agents like *Chlamydia*, *Mycoplasma* and others that cause acute respiratory infections are high-prevalence pathogens, so at the adult age, most of the population already has antibodies in serum-epidemiological assessments. Therefore, the control group individuals were not paired for age, as in the adult age, it is assumed that there is no association between the serologic values and age range. Thus, when choosing younger individuals for this group (blood donors), the possibility of non-clinically detectable CAD was also decreased.

We chose the class IgG immunoglobulin because it has a mean life of 20 to 30 days and it is the antibody (Ab) that better expresses the activity of the infectious process, due to previous re-infections[14].

Charts 1 and 2 show that the patients who presented ACS with ST elevation had a significant decrease in the anti-Cp and anti-Mp Ab titers. This decrease strongly suggests that the Chlamydia and Mycoplasma present in the atherosclerotic plaque had been active, as the occurrence of simultaneous re-infection by the two agents would be very unlikely.

It can be speculated that the co-infection of the atherosclerotic plaque might have been the "trigger" that activated these two agents, starting the inflammatory cascade that, in turn, would lead to the acute coronary event.

In the group without ST-elevation, only a slight, non-significant decrease in the anti-Cp serologic levels was observed, with values that were practically identical in relation to the Mp. These findings suggest that, in this form of ACS, there would be an infectious reactivation of lower intensity, leading to lesser atherosclerotic plaque damage.

When we compare the four groups, using the samples collected at the first phase of the study, one can observe higher serologic values in the groups that presented acute coronary event when compared to the other two groups (chronic atherosclerosis and controls, regarding the anti-Cp as well as the anti-Mp Ab). However, the differences were not statistically significant.

Additionally, no significant differences were observed regarding the serologic values for Chlamydia as well as for Mycoplasma between the chronic atherosclerosis and control groups. These data suggest that the participation of the infectious process can be restricted only to the plaque destabilization mechanisms. Finally, similar results were demonstrated between the serologic values found in the control and chronic atherosclerosis groups; however, one cannot rule out the possibility (in our opinion, a remote one) that this occurred because the control group already had an ongoing atherosclerotic process.

It becomes difficult to compare the results of the present study and the other serum-epidemiologic studies, as there are important methodological differences between them. The majority of these studies was retrospective,[3,5, 15-17] and the few prospective ones used varied Ab measurement techniques and different criteria for serologic positivity[4,6,18].

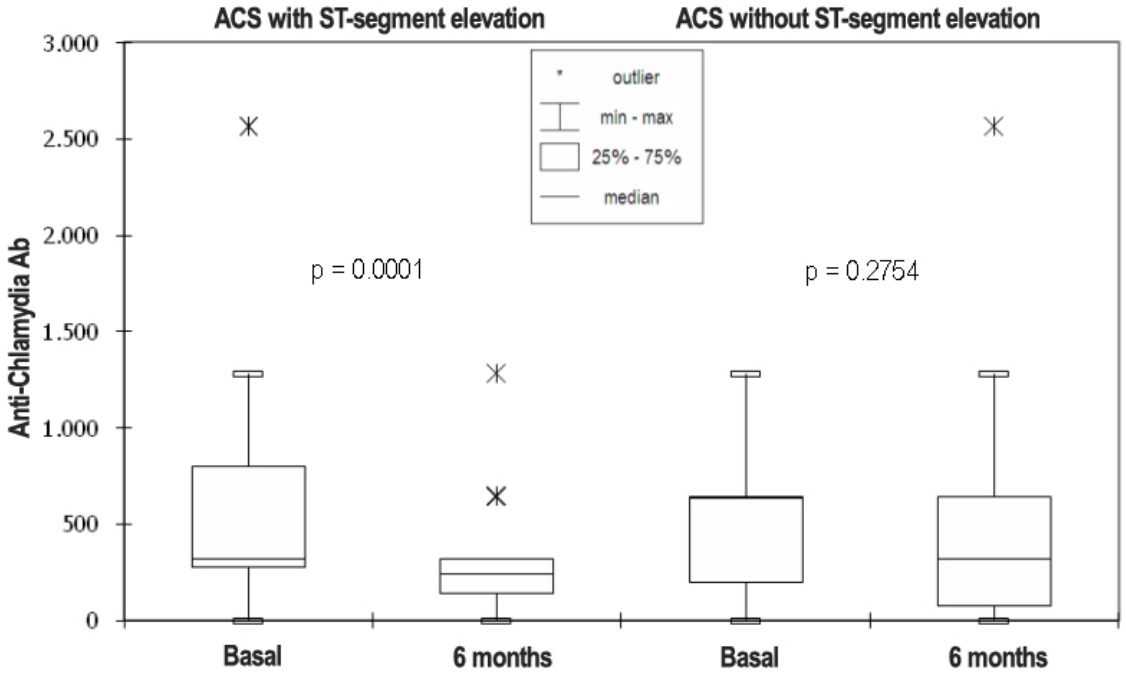

*Chart 1 - Comparison between the Anti-*Chlamydia *serologies obtained at hospital admission due to ACS and 6 months after hospitalization; ACS – acute coronary syndrome; Ab – antibodies; IgG anti-*Chlamydia *antibody titers measured by indirect immunofluorescence.*

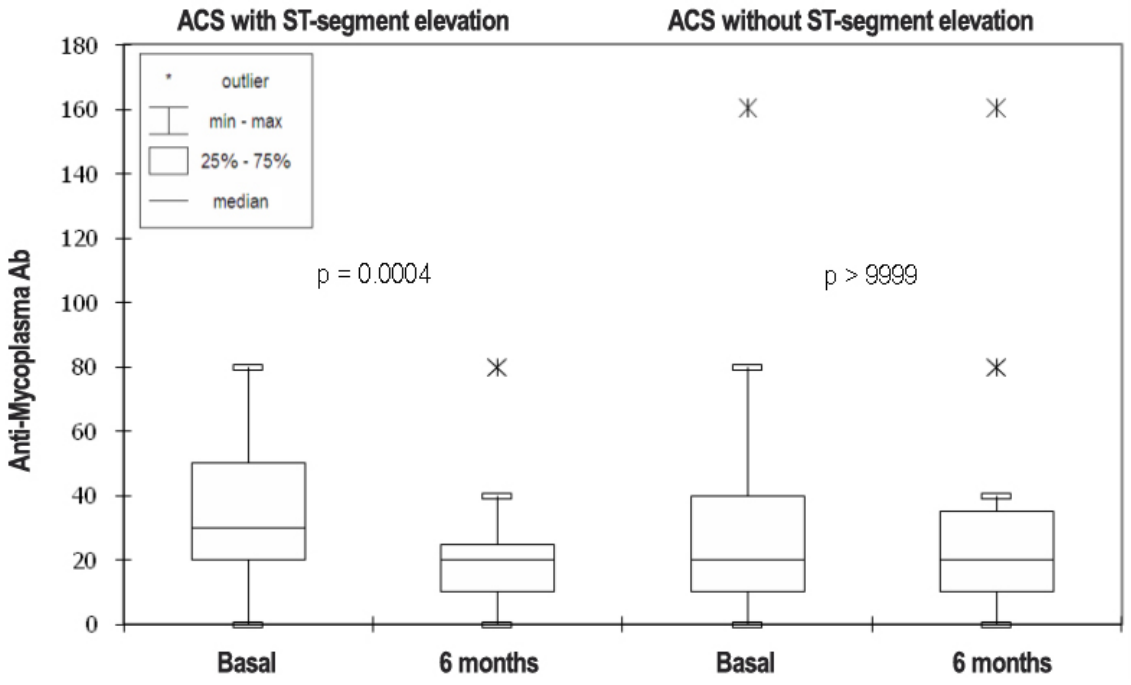

*Chart 2 - Comparison between the anti-*Mycoplasma *serology obtained at the admission due to ACS and 6 months after the hospital admission; ACS – acute coronary syndrome; Ab – antibodies; IgG anti-*Mycoplasma *antibody titers measured by indirect immunofluorescence.*

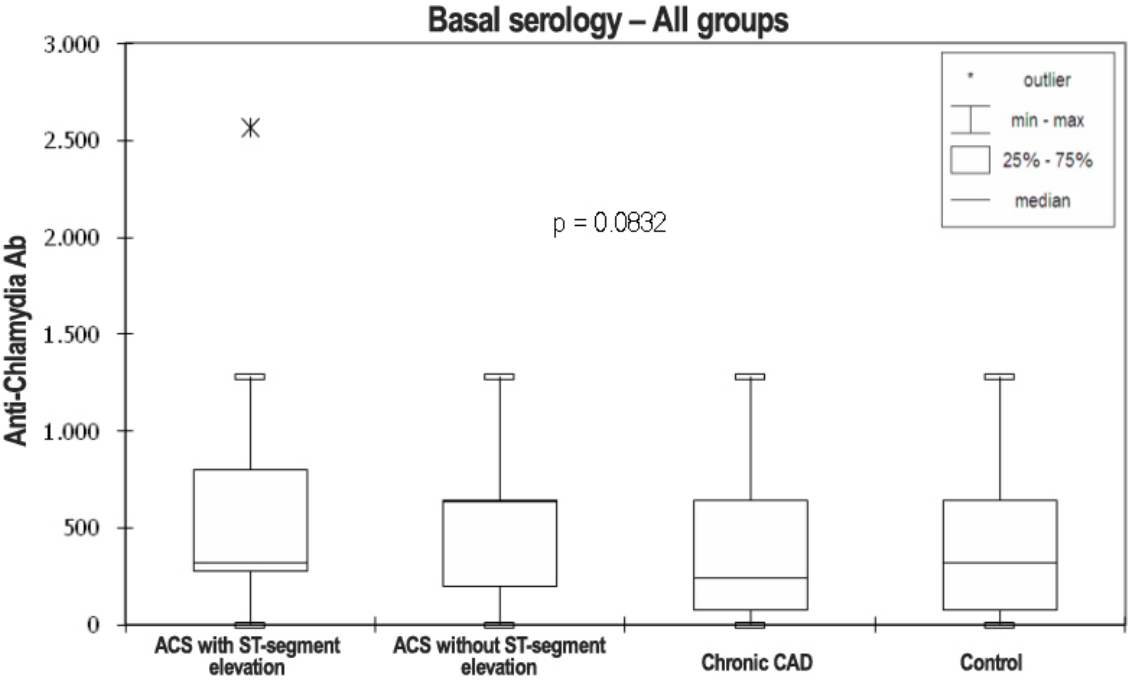

*Chart 3 - Comparison between the basal serum levels of Anti-*Chlamydia *antibodies of the different analyzed groups; ACS – acute coronary syndrome; Ab – antibodies; CAD – coronary artery disease. IgG anti-*Chlamydia *antibody titers measured by indirect immunofluorescence.*

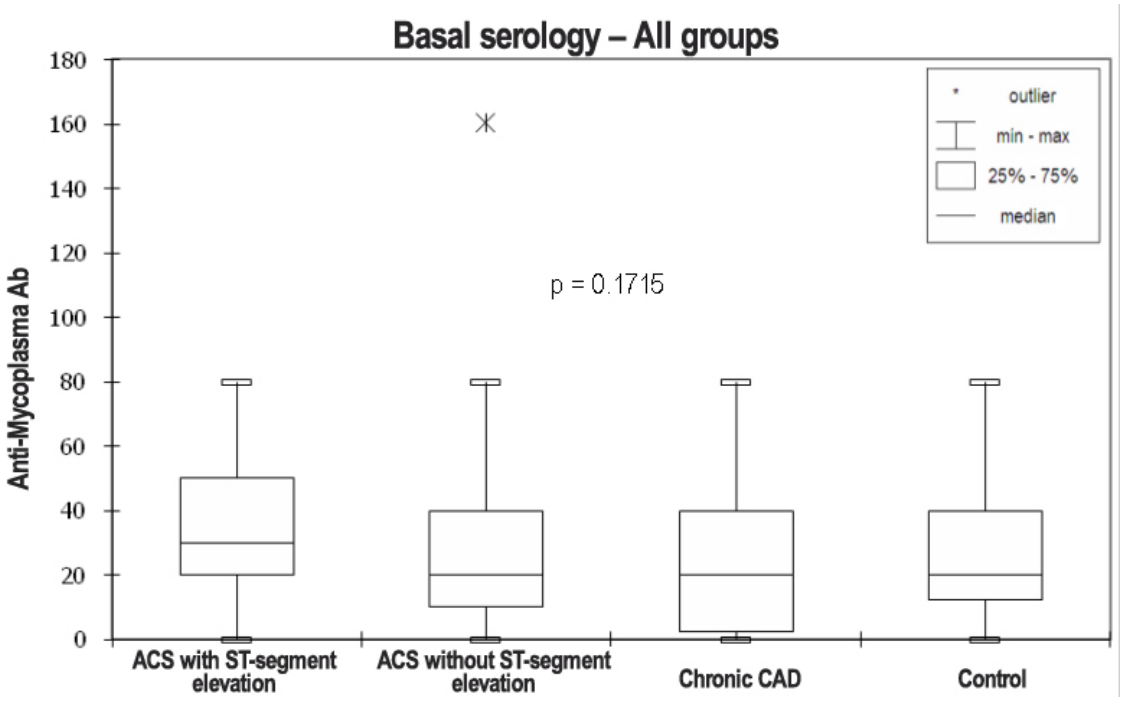

*Chart 4 - Comparison between the basal serum levels of Anti-*Mycoplasma *antibodies of the different analyzed groups; ACS – acute coronary syndrome; Ab – antibodies; CAD – coronary artery disease. IgG anti-*Mycoplasma *antibody titers measured by indirect immunofluorescence.*

On the other hand, some of these studies demonstrated that a high load of pathogens, when acting simultaneously in the same individual, could exacerbate the atherosclerotic process[19,20], which is in disagreement with the findings of the present study.

Conclusions

The present study demonstrates an association between titers of anti-Cp and anti-Mp antibodies and acute coronary disease. It also demonstrates the normalization of the titers within a six-month period, starting from the patient's clinical destabilization.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

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